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INDEX NUMBER
THIS NUMBER COMPLETES VOLUME XLVIII

Vol. XLVIII DECEMBER, 1943 No. 6

THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL
AMERICAN TRUDEAU SOCIETY

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PUBLISHED MONTHLY
at Mount Royal and Guilford Avenues, Baltimore 2, Md.
by the
National Tuberculosis Association
Business Office, 1790 Broadway, New York 19, N. Y.

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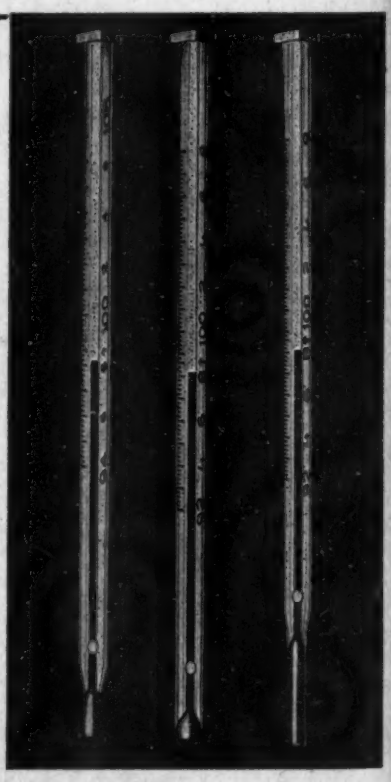

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THE BEGINNINGS OF THE AMERICAN ANTITUBERCULOSIS MOVEMENT[1]

JAMES ALEXANDER MILLER[2]

The beginnings of the American antituberculosis movement may be conveniently, if not too exactly, fixed at the turn of the last century.

This movement is of historical interest, not only because of the effect which it has had upon the control of one of the most wide-spread of diseases, but also because, to a large degree, it served as a pattern for many other similar efforts in the field of social and preventive medicine.

Furthermore, this movement represented one of the first organized efforts to popularize the advances of medical knowledge and thus to unite the combined forces of scientific and lay education into a coöperative effort quite foreign to the attitude of dogmatic aloofness which had hitherto characterized the relations of physicians to the public.

Finally, the story of those early days has a peculiar fascination because of the medical personalities which were involved in it. Diverse in their experience and backgrounds, varied in their abilities and interests, heterogeneous in their personal characteristics, they formed a dynamic and colorful group which gave leadership to a movement which paved the way for much of our public health program as it exists to-day.

It must not, of course, be imagined that this American movement was in any sense an original New World idea.

During the period which we are considering, American medicine was, as never before or since, under the spell of European influence and so it was in the field of tuberculosis. Particularly in Germany, but also in Scandinavia, France, Italy and England, the lead had already been taken and it is to be noted that many of the American leaders were those who were in contact with and impressed by this influence from abroad.

Also it is not surprising that although, with few exceptions, these leaders in no sense had made tuberculosis their especial interest, yet they made this particular disease the object of their first united effort because it was known to be an infection, thus appealing to the upsurge of the bacteriological interest of the times, and also because it presented social, economic and public health implications which challenged their imagination from many directions.

Let us briefly survey the setting in which the scene was laid in the last decade of the past century.

The world in general had been at peace for twenty-five years. In Continental Europe, in Victorian England and in the "Gay Nineties" era of this country, there had been an extraordinary exploitation of the rapidly expanding knowledge in all fields leading to a material prosperity hitherto unknown and to social and

[1] An address delivered before the New York Society of Medical History at the New York Academy of Medicine on April 8, 1943.

[2] 133 East 64th Street, New York, New York.

economic developments which fostered an individual and national self-assurance and complacency very comforting to the body, though not very nourishing to the soul.

In this country, to be sure, the Spanish American War temporarily ruffled the surface but this was later discovered to be merely an episode in our march toward the fulfillment of "our manifest destiny."

In medicine, too, great things were stirring, though not in this country. During the greater part of the 19th Century medicine had been comfortably dozing in the same sleep which had continued for two hundred years, interrupted only by the occasional awakening caused by the dreams of a Laennec, a Henle, a Müller, a Claude Bernard or a Virchow.

But in the seventies things began to move with the arrival of the bacteriological era and with it the dawn of modern scientific medicine.

Pasteur, Lister, Koch, Baumgarten, Roux, von Behring and many others, vying with each other to open the doors of new knowledge in the field of the infections, suggested the means for their prevention and treatment to a new generation or practitioners surfeited with merely empirical medicine.

Inasmuch as these brilliant advances in medical thought all emanated from Europe, it is not surprising that alert young medical practitioners turned there for postgraduate education. Just as in the Colonial days of the 18th Century, it was the returned students from Europe who laid the foundation of early American medicine, so at the end of the 19th Century it was again such returning students, particularly those from Germany and Vienna, who stimulated the beginning of scientific and preventive medicine in this country.

The conditions of American medical practice in the 1890's were in sore need of such a stimulus.

To be sure, some beginnings were being made in recognizing the importance of pathological anatomy as a basis for the diagnosis and understanding of clinical disease and the "dead house" had begun to attract the attention of students under the influence of such leaders as Welch and Prudden in the laboratory and Delafield and Janeway in the clinic. Also Biggs and Park were quick to follow the lead of von Behring in adopting the use of antitoxin in the treatment and prevention of diphtheria and Biggs was attempting to obtain recognition of tuberculosis as a public health problem.

But in general the practice of medicine was a hit-or-miss affair, largely empirical, and its practitioners owed their success mainly to personal qualities, good bedside manners and an extraordinary dogmatism with which they assumed an attitude of "high priests of mystery," whose pronouncements were to be accepted without question and without elucidation.

Intestinal infections were rampant and uncontrolled. The death of thousands of infants each year from summer diarrhoea was the accepted and expected event and typhoid fever claimed almost half the beds in our hospitals during three months of each year. A mass demonstration of the ignorance in sanitation at this time was afforded by the enormous morbidity and mortality from typhoid fever and dysentery among our troops in the Spanish American War.

Medical education also was in a parlous state awaiting the outcome of the newly conceived Johns Hopkins experiment and woefully in need of the house-cleaning which was later to come under the leadership of Abraham Flexner, with his ideas inspired by Germany.

So no wonder it is that tuberculosis, too, continued its devastating way, undiagnosed, uncontrolled and largely untreated.

A death rate of 200 per 100,000 of population in the United States prevailed from this disease and in the larger cities it was nearer 400. The industrial age had brought urbanization with its overcrowding in unspeakably unsanitary tenements, working conditions were controlled only by the profit motive and the reform of social inequities was in its very infancy.

Although the infectious nature of tuberculosis had been known for fifteen years, there were no efforts of any moment either to segregate the diseased or to protect the well. As a public health responsibility tuberculosis was ignored and, though suspected to be a social and economic problem, little was done about it.

Clinically, early diagnosis was almost unknown, as that great revealer of unsuspected lesions, the roentgen ray, had not yet come into its own, and being recognized only in the later stages, tuberculosis was generally considered by physician and layman alike to be a usually fatal disease.

To the student, the disease was of interest mainly because of the many curious sounds that it produced in the chest. These had been brilliantly described by Laennec nearly a century before and they made the tuberculous patient a treasure-house for many succeeding generations of physical diagnosticians, but to the therapeutist he was an object considered unsuitable for or unworthy of serious attention.

My own personal experience will afford a glimpse of the situation as it existed at that time.

In 1903 at the request of the then Professor of Medicine at the College of Physicians and Surgeons, Dr. Walter B. James, I was transferred from the relatively aristocratic surroundings of the Vanderbilt Clinic, to those of Bellevue Hospital. Although my appointment was on the general medical service, my interest in tuberculosis had been aroused by a fortunate personal and professional relationship for two years with Doctor Trudeau in the Adirondacks, who taught me, among very many other things, that my medical school and hospital internship experience had left me totally ignorant of the basic principles underlying a knowledge of tuberculosis. For that reason, in addition to my regular work, I soon began to look into the tuberculosis situation at Bellevue. There was no competition for no one else was or had been interested in the situation.

I found at Bellevue that in the very crowded Out-Patient Department hundreds of cases of pulmonary tuberculosis were included in the general medical clinic. They were almost exclusively advanced cases, in which the diagnosis was easy and the treatment consisted of a prescription for codliver oil and a cough mixture. But they were encouraged to come back regularly for we were a teaching clinic. There was no attempt made to give instruction in the sanitary dis-

posal of the sputum, no follow-up in the homes, no study of their social or economic status and no effort to examine the family contacts.

When they became acutely ill these out-patients were advised to enter one of the overcrowded city hospitals, where they usually died or, if they improved, were discharged to their homes to again visit the dispensary and repeat the same cycle.

Within Bellevue Hospital itself there was a male and a female ward for tuberculosis with a total capacity of 80 beds. This was a transient service, from which within a few days all patients not too sick were transferred to the custodial care of the other city tuberculosis hospitals; the others stayed until they died.

This tuberculosis service was a part of what was known as the outlying services. In addition to tuberculosis they included the alcoholic wards, the prison wards, the erysipelas ward and the medical service to the psychopathic wards, in all comprising 300 to 400 beds.

All of the patients in these outlying services were attended by one single assistant visiting physician, of whom I was one, who was on duty for a period of six weeks, when he was replaced by a similar physician for one of the other three divisions of the hospital and then returned to another six weeks' service in six months' time.

This visiting physician was assisted by one single intern who likewise changed every six weeks. Needless to say, the attention which the patients received was very sketchy and this was particularly so in the tuberculosis wards, for the acute and urgent cases were in the other services and the chronic cases had to wait, often in vain, for many of them were never even examined.

There were standing orders for a cough mixture, for one quarter grain of morphine in case of haemorrhage and a regular half ounce of whiskey three times a day for every patient, with a double portion on the day of their projected transfer to another hospital.

There were two elderly nurses on duty and these were not graduates, for the regular and younger nurses shunned the service, as was the case with both the interns and the students.

Such was the sorry picture, which I am not recounting as a particular aspersion upon Bellevue, but as a record of what was happening all over the country at that time. The romantic story, of course, is that of the steady building up of that service until to-day it is one of the best and most popular in the hospital for physicians, nurses and students alike, but this too has happened all over the country, but, as we take it for granted to-day, we are apt to forget the unhappy beginnings of only forty years ago.

But nevertheless there were some bright spots in the general picture even then.

In 1884 a partly recovered invalid established in the Adirondacks the first sanatorium in this country for the treatment of tuberculosis. Beginning with only a single little cottage, by 1900 it had grown to be a model group of cottages, accommodating over 100 patients who received excellent medical and nursing care. Not content with this, Doctor Trudeau, who had had no previous technical training, set up a laboratory at first simply for the examination of sputa.

but later for experimental researches, especially in the field of immunity toward which he had been stimulated by the studies of Koch and others and by the successful results obtained in other infections, notably diphtheria.

In 1891 Dr. Vincent Y. Bowditch was instrumental in establishing at Sharon, Massachusetts, the first sanatorium situated near a large city and consequently without the climatic advantages then considered as essential.

In 1892 the Pennsylvania Society for the Prevention of Tuberculosis was organized, the first in this country, along the general lines later adopted by the National Tuberculosis Association.

In 1893 Dr. Hermann M. Biggs established in New York the first municipal laboratory as a diagnostic aid in tuberculosis and other infections, especially diphtheria, and thus laid the foundation for the modern public health laboratory now a bulwark of preventive medicine. The municipal research laboratory under Dr. William H. Park was opened the next year.

In 1897 New York City passed the first municipal ordinance requiring the compulsory notification of cases of pulmonary tuberculosis. This was the result of the persistent efforts of Doctor Biggs under whose leadership the New York Health Department as far back as 1889 had officially recognized the fact that tuberculosis was a communicable disease and issued the first educational leaflet on the subject under the title "Contagious Consumption."

Ever since 1893 Doctor Riggs had continued to insist that notification of the disease was an important and proper public health procedure. This suggestion was strenuously opposed by the New York and Kings County Medical Societies and discountenanced by the Academy of Medicine, upon the grounds that it violated the confidential relationship between physician and patient and this opposition continued for a number of years after the adoption of the Board of Health ruling and in consequence the reporting of private cases only very slowly became the general practice, indeed it is still incomplete. It was not until 1907 that the notification of *all* forms of tuberculosis was made compulsory.

In 1902 the Committee on the Prevention of Tuberculosis of the Charity Organization Society was organized in New York City, which in 1919 became the New York Tuberculosis Association.

In 1903 the first independent tuberculosis clinic was organized at the Gouverneur Hospital in New York and the same year the Henry Phipps Institute for the scientific study of tuberculosis under the leadership of Dr. Lawrence F. Flick began its splendid work in Philadelphia, which was destined to have a far reaching influence.

In 1906 the Chicago Tuberculosis Institute was organized under the sponsorship of such leaders in medicine as Dr. Henry B. Favill, Dr. Theodore B. Sachs, Dr. Robert H. Babcock, Dr. Arnold C. Klebs and Dr. Frank Billings.

In the meantime the sanatorium movement had begun in earnest and many of them, both public and private, were established. Among the earlier ones were the Loomis Sanatorium at Liberty, New York, the Eudowood Sanatorium outside of Baltimore in 1896, the Aiken Cottage Sanatorium in 1897, the first State Sanatorium at Rutland, Massachusetts, in 1898, the U. S. Army Sanatorium at

Fort Bayard, New Mexico and the U. S. Public Health Service Sanatorium at Fort Stanton, New Mexico, both in 1899 and the Stony Wold Sanatorium in 1903, this last owing its existence to the enthusiastic persistence of a noble woman, Mrs. James E. Newcomb.

The number had become so great and the special medical interests involved so well recognized, that a clinical society for sanatorium physicians was organized in 1905 under the designation of the American Sanatorium Association, which has continued ever since to have a great influence upon the standard of medical work in these institutions.

All of these various activities in different parts of the country gave evidence of the rapidly increasing interest in tuberculosis and formed a part of the reawakening of American medicine in general. They were, however, localized and sporadic efforts and it was soon recognized that they should be correlated and coördinated if results commensurate with the task of meeting the problem were to be obtained.

In 1899 Doctor Flick suggested the desirability of a national tuberculosis organization but nothing came of it at the time. In the meantime such national societies had been developing in Europe very rapidly and had led to the organization of International Congresses to discuss tuberculosis. Some interested observers here, notably Dr. S. A. Knopf, were convinced that America should hold such a Congress, but, when it was proposed, the lack of any representative body competent to offer an invitation to foreign societies or to arrange a suitable program for an International Congress was considered to be an insuperable obstacle and it was thus that in 1904 a plan for a national association was broached as a preliminary to a later International Congress to be held in this country.

A conference held in Baltimore under the chairmanship of Dr. William H. Welch in January, 1904 was attended by an interested group of prominent physicians and a few laymen. A later meeting was held in Philadelphia in March. As a result a small committee was appointed by Doctor Welch, consisting of Doctor Osler as chairman, Doctor Trudeau, Doctor Biggs, Doctor Welch, Doctor Flick, Doctor Sternberg and Dr. Henry Barton Jacobs, to draw up a constitution for the new society. This Committee proposed the name of "The National Association for the Study and Prevention of Tuberculosis."

The objects of the Association were tersely stated to be "(*1*) the study of tuberculosis in all its forms and relations, (*2*) the dissemination of knowledge about the causes, treatment and prevention of tuberculosis, (*3*) the encouragement of the prevention and scientific treatment of tuberculosis."

The first meeting was called for June 6, 1904 in Atlantic City, at which time Doctor Trudeau was elected President and a large and enthusiastic meeting launched the organized movement against tuberculosis as we know it to-day.

The original Board of Directors consisting of thirty members were all physicians excepting two. These two laymen were Mr. Edward T. Devine and Mr. Frederick L. Hoffman. The physicians were selected from all sections of the country and the list contained the names of many of the most outstanding members of the profession.

A sufficient number of members and financial support were obtained to justify the opening of an executive office in New York in January, 1905, at which time Dr. Livingston Farrand was installed as Executive Secretary of the Association.

Thus began the organized movement throughout the country, which eventually led to the formation of similar organizations upon State, County and City lines, all welded together in the coördinated program for education and prevention as we know it to-day.

In addition to this routine program of propaganda and organization, there were two occurrences in these earlier days which have historical significance. In 1908 the efforts to hold an International Congress in Tuberculosis were crowned with success and a very large and enthusiastic gathering and representation from all parts of the world met in Washington. It was a tremendous success and did much to further the rapid extension of the movement.

The outstanding immediate results of this Congress were development of techniques for popular education through the use of the health exhibit, and even more important was the emphasis which was placed upon institutional segregation of infectious cases as a preventive measure of the first magnitude.

This resulted in the very rapid increase in the number of hospitals and sanatoria for the care of the tuberculous in the United States, which by many is considered the most potent single factor in the rapid decline of the disease. In 1900 there were 4,500 beds for the tuberculous in this country and in 1940 there were 100,000.

The other notable happening was the development of the Christmas Seal Sale as a fund raising device for the support of the movement.

In 1907 Jacob A. Riis noticed a peculiar looking seal upon the back of a letter received from Norway. It was the Norwegian Tuberculosis Christmas Seal and Mr. Riis wrote an article about it in "The Outlook."

Miss Emily Bissell of Wilmington, Delaware, read the article and with newspaper support launched the first Christmas Seal Sale in this country for the benefit of a tuberculosis camp in which she was interested. In this way she raised $3,000 for that purpose.

This encouraged Miss Bissell in the idea of a wider plan and, being an enthusiastic member of the American Red Cross, she offered to finance the project if the Red Cross would sponsor it upon a national scale as a peace-time project for aiding the campaign for the prevention of tuberculosis.

In 1908 the first country-wide Christmas Seal Sale was conducted and realized $135,000. This was a strictly Red Cross enterprise and the seal bore the well-known Geneva cross of that organization and when repeated in 1909 raised $200,000.

Inasmuch as by this time the National Association was becoming well organized, in 1910 Doctor Farrand approached the Red Cross with the proposition of conducting the seal sale as a joint enterprise by the two organizations. It was finally so arranged that the Red Cross continued to sponsor the enterprise and advanced the funds for the expense of the sale and the National Association became the selling agent, each organization sharing equally in the net returns.

During the War in 1918 there was no Christmas Seal Sale and in lieu of that

the Red Cross appropriated $2,500,000 to the National Association for the support of the numerous new antituberculosis agencies throughout the country.

In 1919 for the first time, both the Geneva cross and the double barred cross appeared upon the seal and the return that year was $3,872,622.

In 1920 the American Red Cross relinquished its interest in the Christmas Seal and that year and every succeeding year only the double barred red cross emblem of the antituberculosis crusade has appeared upon the seals.

In 1942 the return for the sale of these tuberculosis seals amounted to over $9,000,000.

The great and too little known debt which the antituberculosis movement owes to the American Red Cross is but another chapter in the record of the notable achievements of that splendid organization, which at the present time is so close to our hearts because of its magnificent efforts to mitigate the horrors of the War.

Thus has the antituberculosis movement been financed and it has spread into every nook and cranny of our land. There are now at least 1,700 organized associations or committees in the United States, all of them directly or indirectly linked with the National Tuberculosis Association.

Opinions may differ as to the relative importance which should be allotted to the many factors involved in the rapidly diminishing incidence and mortality from tuberculosis in this country, yet I think none would deny that much credit must be given to this magnificent organization which has played such an important rôle in implementing the growth of scientific knowledge into practical methods for its prevention and treatment.

There is, however, one broad and profound influence which the tuberculosis movement has exerted which goes far beyond the sphere of one disease. I refer to the momentous change which has occurred in the relation of medicine to the general public.

We have now arrived at a point that through the press and the radio, as well as through the direct approach by individual research workers and physicians, the public is kept informed of the progress of medical science. Their interpretation of this information is, of course, often partial or even faulty, but through the process of general health education it is becoming increasingly accurate, so that a better mutual understanding between physicians and the public is gradually being attained.

It is obvious that the interest in these subjects is very wide-spread and as a result not only does an aroused and more enlightened public opinion make more demands upon individual physicians than ever before, but also physicians are able to coöperate more effectively with their patients.

That some difficulties or even awkwardness have arisen from this situation is true, but it is one that is here to stay and the wise physician will adapt himself to it and endeavor to develop its possibilities to the best advantage of all concerned. As we have seen, a generation ago no such relationship existed but the methods employed in the antituberculosis movement led the way to this change, which now has been extended to all those branches of medicine in which the possibilities of prevention and treatment make them of popular interest.

With all the faults and imperfections involved, it would appear that, on the whole, this relationship of improved mutual understanding constitutes a real contribution to the cause of better public health. But be that as it may, for better or for worse, the leaders of the antituberculosis movement are largely responsible for having started it on its way, and thus initiated another movement even greater than their own, the end of which is not yet.

In our review of the beginnings of the antituberculosis movement, we have referred frequently to its leaders. Inasmuch as the generation who knew them personally is passing, it may be interesting at this time to sketch briefly the personalities of some of these men who so profoundly influenced the history of American medicine.

As the outstanding personality in the field of tuberculosis in America, it is fitting to consider first that romantic figure, Dr. Edward L. Trudeau. This has a particular appeal for me for I was privileged to have a very close personal and professional association with him over a period of many years.

His work and his personality must always be considered against the background of the struggle with his own very extensive disease. As a young man in New York he had been a gay rollicking blade but quite suddenly became serious, took up the study of medicine and started its practice as a genito-urinary surgeon. Almost immediately his disease seized him in an acute form and upon the advice of Doctor Loomis he took the pioneering step of seeking health in the Adirondacks in the dead of winter.

He was very ill and flat on his back for a year and then very gradually improved until at the end of three years he was apparently well and able to take a keen interest in hunting, in which sport he became an expert and to which he was devoted all of his life.

Then again at the end of his life his disease returned in an active form and for over a year before his death he was again a bed-ridden invalid. In the intervening period he accomplished his life work with the handicap of extensive permanent damage to his lungs always with him, aggravated by frequent attacks of excruciating migraine headaches.

When I first knew him in 1901 he was in the midst of this active period. The impression which he made upon me is unforgettable; an enormous head extraordinarily high above the ears, a tall body, gaunt to thinness with extremely long legs set very wide upon the hips, a penetrating eye kindly in expression but dancing with humor, a quickness and energy of movement which belied his apparently frail physique. The brilliancy and vivacity of his conversation fascinated the listener no matter whether the subject might be a scientific problem, a human interest story dealing with tuberculosis, the philosophical and religious aspects of life, the fascination of rabbit or deer hunting and other interests of the woodsman or his delight in flowers, especially if they were red, or the appreciation of the charms of attractive women in whose company he was always particularly happy and even more sparkling than usual.

His charm was irresistible, his enthusiasm contagious. The gradual develop-

ment of his sanatorium was largely due to these qualities. Well over a million dollars came to him for this institution because his own enthusiasm for it was forced upon those to whom he appealed. The same was true for his laboratory and for the countless number of individuals, the knowledge of whose financial predicament he passed on, as occasion arose, to his many more fortunate friends and admirers.

He was similarly personally generous. Many a patient found a $5.00 bill laid on his bed table with the prescription after his visit. He was fortunate in having a fairly competent private means, so that he never took the practice of his profession very seriously as a source of income. As a result his fees were ridiculously small, and the effect was to place Saranac Lake on a professional basis of service rendered, rather than compensation received, which, to be sure, worked somewhat of a hardship upon the younger physicians not so fortunately circumstanced as their chief. This primarily altruistic motive in practice has become characteristic of this community and forms a part of the spirit of Saranac Lake for which the influence of Doctor Trudeau is largely responsible.

He was always eager to help his patients and his inspiration, based on his own personal experience, often was the turning point toward their recovery, but if this was not to be, many was the patient who died philosophically and in peace because his hand was in theirs.

There was a deep spiritual side to Doctor Trudeau's nature, but while he was a regular attendant upon church services, he could not accept many of the orthodox creeds. He used to say that if he ever got to heaven it would be by clinging to Lottie's skirts, for his calm, sweet wife had an unquestioning faith in the religion in which she had been reared, but, if Christianity represents a belief in what is right and pure and is expressed by devoted service to others, then Doctor Trudeau himself was indeed a true Christian.

One day we were driving along the heights above his sanatorium where there is a breath-taking view of the White Face range. He stopped and said, "This is where I wrote my one and only sermon." When I asked its subject he replied, "The Everlasting Hills." This sermon was never delivered or published but when I later read it I found it to be a powerful presentation of the imminence of God in nature, of which any great preacher might well have been proud.

He would discourse by the hour upon the meanings of life and to a young man, as I was then, his philosophy was most inspiring and made a deep and lasting impression.

Even at the height of his great reputation he never understood it and would often say, "I don't know why these people all want to come to me." Yet he was frequently annoyed by some of its consequences. "Never get in the public eye," he said once, "people have no consideration for you, read this." It was a telegram from New York which read as follows: "Am coming to your sanatorium, meet me at the station, arriving 6 a.m."

He was more amused than annoyed by another incident. A man insisted upon his consent to the exploitation of a new five-cent cigar to be called "The Edward

L. Trudeau Cigar." Unable to understand Doctor Trudeau's persistent refusal, he cried out in exasperation, "What's the matter with you? I tell you it's a *good* cigar."

He chuckled over another experience. A man came up from the city to get Doctor Trudeau's opinion concerning his lungs which he had been told were affected. He presented a chest painted all over with iodine on the left side. After examination Doctor Trudeau said, "You certainly have tuberculosis but it is on the right side, why have you painted the left side?" "Oh," exclaimed the patient, "that's what the other doctor said, I wanted to see if you would know!"

With all the serious purpose of his life, Doctor Trudeau had a keen sense of humor and relationships and experiences which presented life in lighter vein gave him great delight.

He had his weaknesses which endeared him all the more to those who knew him. He indulged everybody and everything to which he was deeply attached. He spoiled his horse, his dog and his children.

His Latin temperament was frequently expressed by violent outbursts of irritation but they never lasted long or left any rancor or bitterness behind.

Of the numerous, interesting and well-known characters with whom Doctor Trudeau came in intimate contact during his practice at Saranac Lake, Robert Louis Stevenson is perhaps the most outstanding. Stevenson spent the winter of 1887–1888 there as Doctor Trudeau's patient although he seldom followed his advice. He hated the cold and spent the winter as a recluse cooped up in the modest Baker cottage with all windows tightly closed.

Doctor Trudeau was one of his very first visitors and he was privileged to attend the informal rehearsals of "Dr. Jekyle and Mr. Hyde" by Richard Mansfield which Stevenson supervised in his cottage. One day Doctor Trudeau persuaded him to come out to visit the laboratory. This Stevenson did with great reluctance for he had a very squeamish nature.

After a very few moments his face paled and he made a hasty retreat exclaiming, "Trudeau, your light may be very bright to you, but to me it smells of oil like the Devil."

Of some historical interest may be the fact that Doctor Trudeau privately maintained that he had never been able to detect any tuberculosis in Stevenson's case and he much doubted that Stevenson had this disease which has been so generally associated with his life.

When during the last few years of his life Doctor Trudeau became chronically ill he, like most other doctors, was not a very patient patient. His irritability was often extreme and he did not, by any means, show the resignation and acquiescence which he preached for his patients.

He hated the cold and his room was preferably always kept at a temperature of at least 80°, with all windows closed, and when we went in to visit with him we were almost stifled. We laughingly called it the "Trudeau cure." But even in those sicker days toward the end, his spirit did not leave him and on one oc-

casion he insisted on one last trip to his small hunting preserve with his old guide, and there, carried on a litter and stretched out in a hammock, he shot his last buck. He was a sport to the end.

Doctor Trudeau's minor weaknesses made his greatness stand out all the more in relief and it testified to his humaneness, which was such an outstanding characteristic. His spirit lives on in Saranac Lake and the impress of his personality upon the profession in this country has been very deep.

It is no wonder that, although he was not interested and took no active part in the organization problems of the National Tuberculosis Association, he was elected with unanimous acclaim as its first President.

The next two personalities whom I will sketch fall into quite a different category. Their chief contribution to the movement was in the rôle of forerunners. They are Dr. S. Adolphus Knopf and Dr. Lawrence F. Flick.

Doctor Knopf, as we have already seen, was the chief promoter of the idea of the formation of an International Tuberculosis Congress, the consideration of which led to the formation of the National Association. Doctor Flick in 1899 was the first to urge the need for the national organization of such an association. Both had been active during the '90s in stimulating an increased interest in the problems of tuberculosis.

Although the term was not as familiar in those days as it has since become, Doctor Knopf was a born propagandist. He was not a great clinician and not at all a scientist, but his energy and enthusiasm for popular education were unbounded. In 1900 he published his pamphlet entitled *Tuberculosis, a Disease of the Masses.* This was widely circulated in this country and abroad, being translated into twenty-five languages and it had considerably influence.

During the earlier years of the movement he addressed many hundreds of audiences, mostly of lay composition, on the subject of the prevention and treatment of tuberculosis. His short stature and rotund figure did not lend themselves to dignity but perhaps added to the effect of his vociferous and contagious enthusiasm. Unfettered by the weight of too great scientific knowledge but inspired by his conviction of the importance of his subject, he let himself go with bursts of fiery oratory, which would have made him a successful stump speaker for any cause. He was literally a "spellbinder." He lifted his audience to a pitch of enthusiasm which was truly remarkable when we consider the state of ignorance and lack of interest which prevailed at the time.

Doctor Knopf also had a canny appreciation of the value of prominent names to support his campaign and the fact that he literally pestered well-known physicians with his ideas had not a little to do with their acceptance, at first with reluctance and then with enthusiastic interest, of the responsibility of helping to organize a type of popular movement which at that time was quite new and revolutionary to a conservative medical profession. The naive delight which his association with the leading figures of medicine gave him should not detract from the value of the contribution which he made, for it was truly considerable.

In later years, in 1922 Doctor Knopf wrote a *History of the National Association* to which many beside myself are indebted for the record of its early days.

Doctor Flick was a character of quite another sort. Like Doctor Trudeau, he suffered from tuberculosis which first led to his special interest in the disease and from occasional exacerbations which handicapped him during his long life.

In 1890 he organized the Rush Hospital for Consumption in Philadelphia, in 1892 the Pennsylvania Society for the Prevention of Tuberculosis, the first of its kind in this country which served as a pattern for his conception of a national society, and in 1894 The Free Hospital for Consumption which later became known as the White Haven Sanatorium.

Doctor Flick's most important contribution, however, was the founding of The Henry Phipps Institute in 1903 which was transferred to the University of Pennsylvania in 1909. This institution developed into and to-day remains the outstanding center for scientific and clinical research in tuberculosis in this country and is a permanent monument to Doctor Flick's vision and Mr. Phipps' generosity.

Doctor Flick was a clinician of no mean merit, as well as a real student of medicine, in addition to being a pioneer in the organization against tuberculosis. His career, however, was marred by a difficult temperament which interfered seriously with his usefulness. In the organization of the Congress of 1908, as Chairman of the Committee on Arrangements, he differed violently with others in matters which appear to have been relatively inconsequential and as a result withdrew from active participation in the affairs of both the Congress and the National Association.

Similarly he had difficulties in the administration of the Phipps Institute which led to its transfer to the University of Pennsylvania in 1909 and to his resignation from the Pennsylvania Society and the White Haven Sanatorium.

He lived on in semi-retirement for many years until 1937, but in 1925 he published an important and scholarly book on *The Development of our Knowledge of Tuberculosis.*

It is a great pity that the movement thus early lost Doctor Flick's active participation in it but we cannot forget our great obligation to him as one of its most important forerunners.

The next two leaders whom we will consider were preëminent in the field of public health. They are Dr. Herman M. Biggs and his associate, Dr. William H. Park.

Doctor Biggs' career is well known as first chief medical officer of the New York City Department of Health and later as Commissioner of the State Health Department. Some of his contributions to the tuberculosis program in New York City have already been indicated.

It is perhaps less well known that he was a leading clinician in diseases of the heart as well as of the lungs and that he was Associate Professor of Medicine at New York University and Bellevue Medical College for many years. He was one of the most unassuming but at the same time powerful influences in the National Tuberculosis movement. He was one of the wisest men at the council table that I have ever known and the basis of his wisdom was a scientific approach exercised with practical common sense.

While his activities were predominantly in the field of public health, he was intensely interested in and helpful with the problems of nonofficial agencies, such as the Rockefeller Institute and International Health Board, The Milbank Fund Health Demonstrations and the National Tuberculosis Association.

Curiously enough a considerable part of his success was due to political associations, notably with his devoted friend and patient, Charles F. Murphy, who was the leader of Tammany Hall. Doctor Biggs was no starry-eyed reformer but a practical realist who turned conditions as he found them to his own purposes with uncanny skill. He was deliberate in forming judgments but persevering and tenacious in their execution. He was very quiet and deliberate in his discussions but unerringly recognized the core of a knotty problem.

He never wasted energy for he had little strength to waste, as he was far from robust and suffered from diabetes for many years of his most active life.

Doctor Biggs was one of the first in this country to recognize the importance of preventive medicine, its fundamental dependence upon scientific knowledge and the necessity of its implementation through public health agencies.

Under the influence of Doctor Welch, Doctor Biggs early became intensely interested in pathology and particularly in the new science of bacteriology and thus came in contact with Doctor Park who had been converted from a nose and throat specialist into a budding bacteriologist and was making important studies in diphtheria at the Willard Parker Hospital.

Doctor Park, who was then working in Doctor Prudden's laboratory, was persuaded to join Doctor Biggs in the organization of the diagnostic and later the research laboratory of the Health Department in 1893 and 1894.

It was then that I first met them. I will never forget the occasion, for it was the turning point in my professional life and the beginning of two very intimate and lasting friendships.

By appointment I called one autumn evening in 1894 at Doctor Biggs' office on 58th Street next to the old Plaza Hotel. Doctor Park was charming, gracious and sympathetic, putting me quickly at my ease. Doctor Biggs was reserved and undemonstrative but his piercing black eyes were sizing me up with quiet but obvious interest.

He told me of their plans for research and the need for a chemist in the new laboratory and opened up an entirely new vista of the possibilities of science as applied to public health. I explained that I knew nothing of the problems involved and that my experience had been solely in inorganic chemistry in the industrial field. He replied that he and Doctor Park also knew very little but that they hoped to learn and would I not like to try to learn with them?

At the prospect of association with two such teachers, my dismay turned to ambition and hope. At the close of an hour of fascinating conversation,Doctor Biggs quietly said, "I think you might as well take the examination." I did not realize until later that this meant that my appointment was already assured and if I needed any further proof it was afforded by the realization of the absolutely rotten examination paper that I turned in.

Things were done that way then. For example, I was living in New Jersey

and a New York residence was required to qualify for a civil service position. Doctor Park smilingly gave me an address where I rented a room, hung up an old pair of trousers in the closet and never returned until three weeks later after I was appointed Research Chemist, when I paid my bill, collected my pants and returned to my home in New Jersey, from which I commuted regularly during my term in the city's service.

As director of the laboratory, Doctor Park was a very easy boss but gave freely of his imaginative thought to the management of my chemical problems, but especially of his affectionate personal interest.

Within a year I hesitatingly put this to a severe test. I explained to him that I thought that, in the field of physiological chemistry, a medical education was necessary and that I would like to try a year of medical school but could not afford it. I had discovered that the only course requiring personal attendance was one term of histology in addition to the all important anatomical dissection. Could I have three afternoons off a week for one term, do my dissection at night and keep my job?

He very promptly consented and thus the whole direction of my life was changed.

Doctor Park had very unorthodox ideas of administration, and rules and regulations meant little to him but he held his staff by bonds of the deepest affection which were much more effective than discipline.

Throughout their lives these two men, Doctor Biggs and Doctor Park, were my close associates and were ever ready and helpful personal advisers as they were also to hundreds of others.

They brought science into public health by their own contributions but they were aided greatly by the enthusiastic support of their staffs and coworkers whom they held quite as much by ties of affection as by those of respect for their attainments.

For the next two figures in our story we turn from New York to Baltimore.

Dr. William H. Welch and Dr. William, later Sir William, Osler. Viewed from any angle these two names stand out preëminently in the history of American Medicine. It is significant that they are also associated prominently with those early days of the antituberculosis movement. Our very best were in it.

It would be superfluous to trace their notable careers which are so well known, but their association with the campaign against tuberculosis and a few sidelights on their personalities may be not without interest.

As we have seen, both were members of the original Board of Directors of the National Association and were very active in its organization. Doctor Welch was elected President of the International Congress of 1908 but was unable to serve but he did act as president of its important section in Pathology and Bacteriology. Of the two, Doctor Welch had a much broader and more sustained interest in tuberculosis as a public health problem and in the formulation of the program for its control upon a scientific basis. In this country he took an active part in this direction, not only through the National Association but also in the

American Red Cross and as a member of the Technical Advisory Board of the Milbank Fund in its tuberculosis demonstrations in New York State. Also he was the guiding genius in the establishment of the School of Public Health at Johns Hopkins, which has played a very important part in the development of Public Health administration in the United States.

In the international field he was very influential in the International Health Board of the Rockefeller Foundation, in the International Red Cross and in the Health Section of the League of Nations. So that, in addition to being a recognized leader in his special field of Pathology and Bacteriology, he became an outstanding national and international figure in the field of public health.

But, if possible, Doctor Welch's personal qualities were even more noteworthy.

His varied interests during his long and very busy life brought him in contact with an extraordinarily large number of physicians and there are many living to-day who knew and remember him well. His portly figure, his serene countenance, his perfectly bald head and his twinkling blue eyes rise up in our memories as we think of him. But we remember especially his dignified demeanor, his gracious manners, his clarity of thought and his marvelous facility of expression. His extemporaneous discourses were as great a delight as his more formal scientific presentations were an intellectual treat.

In his bachelor home, however, everything was not so systematic or orderly. Unguided, or if you like, unfettered by feminine control it ran its own natural course which is the way he wanted it. To visit him there gave the keenest pleasure but to have had to live with him in it would certainly have been difficult.

If you wished to communicate with him you would have to see him, for he held to the theory that in time letters answered themselves, so that he never bothered to answer them and his system was to allow them to accumulate upon his desk and, when the pile became inconveniently high, it was neatly covered with newspapers and a fresh layer started; this in turn was covered with newspapers and the process repeated. When a sufficiently bulky accumulation was reached the whole pile was swept off into the rubbish can.

Thus it came about that his friends did not write but went to see him and, if he was interested, time or other engagements were not considerations that could interfere with the interview which was always a precious and stimulating experience for those to whom the privilege was granted. He was as lovable in his casualness as he was inspiring in his brilliancy.

So "Popsy" Welch lived his life, beloved and revered by all and left an impression upon the medical history of this country which is second to none.

Doctor Osler was a personality of quite another sort.

It is told of Sargent that when he was working upon his famous group portrait of the four founders of the Johns Hopkins Medical School, he exclaimed, "Welch is easy, it is like copying a Raphael cherub, but that Osler, he is impossible, unpredictable and never the same. I can't get him at all!" And so he was.

Osler was preëminently known as the great clinician and teacher, but as we have seen he was also interested in public health organization. This was particularly so after he went to England, where he was a moving spirit in many

antituberculosis societies. His interest in tuberculosis began when he was at McGill where he studied many cases at autopsy and as early as 1883 he suggested the possibility of the transmission of the disease from animals to man.

During his few years at the University of Pennsylvania, 1884 to 1888, he spent much time in "Blockley" and later was much interested in Doctor Flick's efforts to organize antituberculosis activities.

It was, however, at John Hopkins, from 1888 to 1905, that Doctor Osler was in the flower of his career and, in addition to his contributions to the knowledge of all branches of internal medicine, he maintained a special interest in tuberculosis.

In his *Text Book of Medicine*, a medical classic, his chapter on *Tuberculosis* was considered by many to be his best and in his organization of the Laennec Society for the study of tuberculosis for a selected group of students, he did much to further the undergraduate study of this subject.

Stimulated by Doctor Trudeau's classic experiment in the results of free range in the open air upon the course of the disease in infected rabbits, Doctor Osler was one of the very first to preach and put into practice the doctrine of the out-of-door-life at home, independent of special climate, in the treatment of tuberculosis and in 1899 published a paper on *The Home Treatment of Tuberculosis.* Also as early as 1898 he inaugurated a system of home supervision and instruction of dispensary patients by visiting nurses.

During this period he published numerous papers on various problems of tuberculosis and thus added much to a better understanding of the disease. Not all of these contributions were strictly medical, notably his address in Philadelphia in 1903 upon *The Home in Its Relation to the Tuberculosis Problem.*

Doctor Osler had many interests beside medicine. He was a bibliophile and book collector of great zest and rare acumen, he was a philosopher as demonstrated by several of his delightful essays and he had a keen interest in human relationships which developed a rare gift for friendship.

His patients, students, friends and associates were devoted to him with an enthusiasm and affection which is rarely accorded to any man. He was keen, witty, vivacious and wise. His sparkling personality was irresistible but his very spontaneity sometimes got him into trouble.

The most famous of these incidents occurred when he rather carelessly, in attempting to emphasize an element of truth, suggested that not only did original productive effort cease at the age of forty but that at sixty one might as well be chloroformed. The press and the public took up seriously this obviously intended exaggeration and for many years it caused him much annoyance and some real concern.

Also he was an inveterate practical joker, often going to considerable pains to perpetrate a hoax. His connection with one of the most famous of these he never admitted directly. This was what has been termed the Egerton Y. Davis tradition.

It started while Doctor Osler was still in Montreal. To the editorial office of one of the medical journals which he was accustomed to frequent, there came at

intervals interesting and extraordinary case reports signed by Egerton Y. Davis, M.D., formerly surgeon in the U. S. Army, who represented himself to be practicing among an isolated tribe of Canadian Indians. Outside of these reports nothing apparently was known about Doctor Davis but his experiences were so colorful and unusual that a number of these reports were published in the journal.

The nature of these reports became increasingly fantastic and culminated in one entitled *An Extraordinary Case of Immaculate Conception*, in which the course of a stray rifle bullet played the miraculous rôle. Suspicion of the authenticity of these reports had already been aroused and was pointed toward Doctor Osler. He admitted that he met Doctor Davis once and only once, that he thought that he was a disreputable character, not to be trusted and advised against the publication of the report.

Later when he was in Philadelphia, Dr. E. Y. Davis again cropped up in a communication to the editor of the Medical News and Doctor Osler was again questioned. He always avoided any confession of responsibility but admitted that he sometimes used the name of E. Y. Davis when registering at hotels when he did not wish his identity to be known. He was always very gleeful about it and reiterated his opinion that E. Y. Davis was an extraordinary but disreputable character.

The last time I saw Sir William Osler was during a weekend visit to his home in Oxford in 1918. After dinner I rallied him about the E. Y. Davis affair and told him that a strong suspicion remained in the minds of his friends that he and Davis were one and the same person. He burst into a gale of laughter and with gleeful zest brought out a number of pamphlets and notes dealing with E. Y. Davis, among them his own note on the subject which Doctor Cushing has published in his *Life of Osler*. He chuckled over these with me for an hour, never admitting or definitely denying my suspicion but ended up with the exclamation, "So they still think that I was E. Y. Davis; that is the biggest joke of all!"

He was a playful boy to the end and I think greatly relished getting back into this mood as a relief from the sedate and dignified atmosphere of the Regius Professorship at Oxford and of his great responsibilities in the War.

This side of his nature merely serves to demonstrate how human he was and to set off his greatness, for he was a truly great man.

How fortunate indeed was the American antituberculosis movement to be able to enlist the deep interest and active support of these two great men from Johns Hopkins, Doctor Welch and Doctor Osler!

In the case of the last personality which I will present to you, as in that of the first, Doctor Trudeau, my presentation is deeply colored by many years of intimate association and friendship.

Dr. Livingston Farrand, as already indicated, was the first executive officer of the National Tuberculosis Association and its rapid development and success were largely due to his skilful and efficient direction. As he went on to fill many

more important and responsible positions, President of the University of Colorado, Director of the Rockefeller Tuberculosis Commission in France, Director of the American Red Cross and finally President of Cornell University, he never wavered in his deep interest in tuberculosis and in public health.

His most concentrated work in tuberculosis was in France. There, with the backing of the International Health Board of the Rockefeller Foundation and with the era of good feeling toward America engendered by the War, he was able to set up an organization for the control of tuberculosis which was astonishingly effective and after the War endured as perhaps the only permanent result of the great American effort in that country.

This was considered by the French themselves to be a most difficult task, for as they expressed it, it was almost impossible to get two Frenchmen to agree to work together on anything. It was a rare treat to watch Doctor Farrand handle politicians, physicians and volunteer workers alike, gradually making it clear that the Commission was not a hand-out agency but a group interested only in sound organization based on modern principles and American experience.

We found the French physicians splendid clinicians and thoroughly grounded in theoretical knowledge but entirely lacking in the practical application of that knowledge in any organized way.

When their great native intelligence came to appreciate what Doctor Farrand was after and that he meant business, their leaders gradually took up his plans with enthusiasm and coöperated splendidly to adapt American ideas to French conditions.

The process, however, was slow and called for skilful diplomacy in which Doctor Farrand proved himself to be adept. The French came not only to admire his efficiency but to conceive a real affection for him on account of the way he handled himself and his job. For they firmly believed in the old French adage, "It isn't so much what you do that matters, as it is the way you do it." It is an art to adapt Yankee directness to the Gallic love of polite indirectness and this is what Doctor Farrand achieved.

His success was due quite as much to his patient understanding as it was to his knowledge and executive ability. It took much patience to sit through innumerable two hour formal *dejeuners*, whose sole purpose was to discuss important problems and to realize that to broach the subject uppermost in all minds would be a breach of etiquette insulting to the cuisine and to the wines and fatal to the future success of the conference. Doctor Farrand learned not only to take this custom in his stride but I think came very much to like it.

When he returned to America as head of the Red Cross, he supported its peace program in public health, one feature of which was the East Harlem Health Center in New York City which was the forerunner of the Milbank Fund Demonstration Centre in New York City and State and laid the foundation for the numerous district health centres in the city which has been such a notable contribution to organization of public health administration along modern lines. Later, as a member of its Technical Board, Doctor Farrand took a leading part in the direction of the Milbank Fund Demonstrations.

He was clear in thought, convincing in debate and forceful in action. As a presiding officer over any meeting large or small, he was without a peer and, without laying claim to oratory as such, his many public addresses were notable for their sound content, logical thought and forcible delivery.

As a personality he was unusual. His slight frame was packed with dynamic energy; his general knowledge and clear thinking made him a leader in any group. Moreover he was always the charming gentleman with a keen sense of humor which made him a delightful companion.

The range of his acquaintanceship was extraordinarily large and the number of his devoted friends was legion. He appreciated and loved the amenities of living which gave an intensely human touch to the more sober side of his character and this made him lovable when his strength might easily have rendered him austere.

He was a member of the Class of '88 which has the reputation of being the most boisterous, fun-loving class that ever graduated from Princeton and "Tony" Farrand was one of its ringleaders. It was perhaps this side of his nature which rendered him proof against any self conceit from his success. He had less "side" than almost any man I have ever known. This was well exemplified during the dignity and pomp of the ceremonies attending his installation as President at Cornell. Amid it all he spied one of his Class of '88 cronies in the crowd near him and, apparently feeling rather sheepish in such company, he turned aside to his friend and hoarsely whispered, "Don't it beat hell!" Anyone knowing him would immediately recognize that this was exactly what he actually felt.

"Tony" also loved good living. Conviviality without overindulgence was a habit of his life and added much to his charm.

The day before he died I was in his room with his physician who asked if I could suggest anything more to do for him. In a firm tone I said, "Yes, give him a good drink." "Tony" looked up at us with a half wink and crooked smile and said, "Don't pay any attention to him; he knows me too well." He had his drink and the next day he died. He was a gallant spirit to the last.

He was one of the stalwarts in the antituberculosis movement and he has left us so recently that the ache for what we miss in his going is still poignant.

We bring to a conclusion this revival of memories of a generation ago. Whatever interest may lie in the story itself, the chief service that the history of the past can render is to provide a better understanding of the present.

The antituberculosis movement originated at the time and was a part of a momentous period in the history of medicine. One of its important phases was the change in the relationship between the public and the medical profession and we have seen how this developed largely under the statesmanlike leadership of physicians themselves.

To-day we live in another critical period in which this same relationship is of very significant importance. In part at least, the trend toward popular health education begun forty years ago with tuberculosis as its focal point, is responsible for the present situation. The significance of better health is now appre-

ciated by a large percentage of the population from organized government down to the individual citizen.

This has led to criticisms of many present conditions and numerous suggestions for their improvement. In a reversal of the previous situation the pressure now comes from the public and is being applied to the medical profession.

Wise men, both within and outside the profession, realize that great social changes are at hand in which physical as well as economic welfare is involved and that consequently many readjustments are necessary. It will not be sufficient to point to the great achievements of recent years which have brought America from near the bottom to the very top in world medicine. The obligation is to utilize this very proficiency to bring about still further progress until the goal of making scientific medicine available to all for the maintenance of health and the treatment of disease has been attained.

The day has gone by when the individual relationship between physician and patient is the sole responsibility of our profession. Our horizon has become immeasurably widened so that many other conditions involving social and community problems are now included. In the solution of these problems physicians must take the lead if we are to avoid serious mistakes, particularly in the sacrifice of quality to the demand for increased quantity of service. Our special training gives us the advantage of better knowledge; we only need the vision to apply it wisely and effectively.

Perhaps our review of the part which physicians played in our early health movement may bring encouragement and inspiration to meet the even more serious situation which confronts us to-day. Inasmuch as whatever comes we must hold fast to the high ideals of our profession, in closing I would leave with you a message from one of the great leaders whose lives we have already reviewed. A few years ago I presented this same message to the New York Academy of Medicine. I trust you will pardon its repetition upon this occasion.

In 1910 Doctor Trudeau, who was already beginning to suffer from a return of the symptoms of his disease, was elected President of the Congress of Physicians and Surgeons. On the evening of his presidential address the large hall in Washington was packed to the doors with a deeply attentive and expectant audience. With the background of his lifelong struggle with difficulties and physical disability and suffering as he was at the time from weakness and a low fever, the very title of his address represented his challenge to fate and the triumph of his spirit. It was *The Value of Optimism in Medicine.*

At the conclusion of that memorable address, he left this message, which is the one that I would leave with you.

"Let us not," he said, "Let us not, therefore, quench the faith nor turn from the vision which, whether we own it or not, we carry, as Stevenson's lantern-bearers their lanterns, hidden from the outer world, and, thus inspired, many will reach the goal; and if for most of us our achievements inevitably must fall short of our ideals, if when age and infirmity overtake us 'we come not within sight of the castle of our dreams,' nevertheless all will be well with us; for, as Stevenson tells us rightly, 'to travel *hopefully* is better than to arrive, and the true success is in labor.' "

ANALYSIS OF BREATHING PATTERN[1, 2]

J. L. CAUGHEY, JR.[3]

There are many problems connected with breathing. For practical reasons, study of the subject is divided into two sections, respiration and ventilation. Under respiration are included all the physico-chemical steps involved in the absorption of oxygen from the alveoli, its transport to and utilization in the tissues, the removal of carbon dioxide from body cells and transfer of it from the blood into the alveolar air. Ventilation refers only to the movement of gas in and out of the alveoli through the air passages in response to the activity of breathing muscles. Respiratory phases of the breathing problem have been the subject of extensive and detailed study. They are outside the scope of this paper, which is concerned only with the ventilatory behavior of human subjects.

Previous work in this field has been principally of two types. One group of investigations has been designed to study the rate of breathing, the depth of individual breaths and the volume of air moved per minute when the subject is at rest (1), or is tested under varied conditions of temperature, barometric pressure or physical exertion (2). The other large group of studies has been concerned with the changes in ventilatory mechanics caused by disease, particularly those of the heart (3), lungs (4) or nervous system (5).

Relatively little attention has been paid to the fact that healthy persons vary greatly in their manner of breathing (figure 1). Early investigators of ventilation commented on the individual differences they saw in the way subjects accomplished the necessary intake and output of air from the lungs and predicted that analysis of these breathing patterns would give information of clinical interest (6). But subsequent studies along these lines have been incomplete and unconvincing (7) and have not led to any practical use of spirographic analysis.

The work of the Constitution Clinic is based on the idea that the total personality of the patient expresses itself in every one of his behavior patterns (8). Therefore analysis of each individual characteristic assumes importance because it contributes one fragment of information about the total quality of the person. For this reason a study of the way people breathe was undertaken, with the hope that eventually this kind of analysis will provide material for significant correlation with other aspects of the total personality of the individual.

This paper presents a method for studying spirograms and the results obtained from applying this method to a large series of tracings.

[1] From the Constitution Clinic, Presbyterian Hospital, and the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York.

[2] This work has been aided by grants from the John and Mary R. Markle Foundation, and from the Rockefeller Foundation.

[3] Associate in Medicine, Columbia University, and Assistant Physician, Presbyterian Hospital. Formerly Walter Belknap James Research Fellow in Medicine, Columbia University.

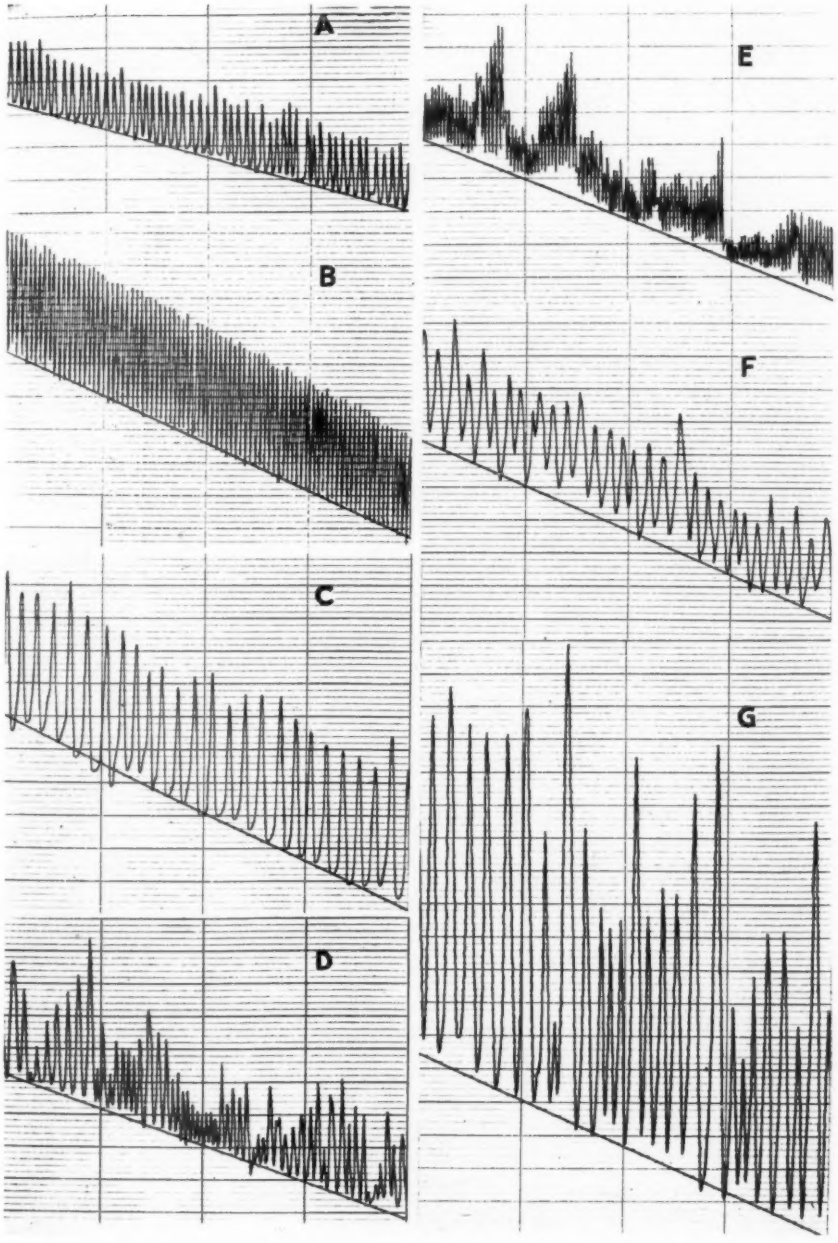

FIG. 1. Tracings to illustrate differences in breathing behavior. The patients were under basal conditions. A Benedict-Roth metabolism apparatus was used. Each spirogram begins at lower right corner. Inspiration is recorded by an ascending line, expiration by a descending line. Horizontal ruled lines are separated by a space of 2 mm., which is equivalent to movement of 41.4 cc. of gas in or out of the machine. Vertical ruled lines are separated by the distance through which the kymograph moves in one minute.

MATERIAL AND METHODS

From the files of the Basal Metabolism Laboratory of the Presbyterian Hospital the spirographic records of 500 female and 200 male patients were obtained. Because of repeated metabolism tests by some patients, the number of breathing records examined was 704 and 280 respectively, a total of 984. No effort was made to select special cases, but persons less than sixteen years of age and patients with obvious cardiac or pulmonary insufficiency at the time of the tracing were excluded from the series. The group represents not "normal" persons, but a cross section of adult patients sent for basal metabolism tests by physicians in all departments of the hospital.

Most of the respiratory tracings were made with the Benedict-Roth metabolism apparatus, but in some a Sanborn graphic machine was used. In all calculations of ventilatory volume made from these latter records, a correction factor of 1.072 was used to convert the figures to the dimensions of the Benedict-Roth spirograms.

In study of ventilatory behavior there is an important problem of terminology. Most previous workers have reported their results as cubic centimeters or liters of gas movement. This has serious drawbacks because volumes expressed in this way are not related to the size and sex of the individual subject. The situation is comparable to that which would exist if metabolic rates were reported as cc. of oxygen consumed per hour, without reference to the sex and size of the patient. Inspiration of 500 cc. is quite a different effort for a small woman than it is for a large man.

One ventilatory measurement in common clinical use is vital capacity of the lungs. Physicians are familiar with this volume because of its relation to the symptoms of cardiac and pulmonary disease. They know that a small woman may have a vital capacity of 3,000 cc. or less, while a large man is expected to have 5,500 cc. or more. It is also well known that vital capacity may be predicted for an individual on the basis of sex and body surface. There is relatively little disagreement among students of the subject about the standards for such predictions (9). These disagreements are unimportant clinically and are not great enough to be a serious drawback to the use of estimated vital capacity as the basis for relation of ventilatory volumes to the size and sex of the individual. The vital capacity of women has been shown to be close to 2,000 cc. per square meter of body surface, while that of men is approximately 2,500 cc. per square meter. A chance relationship between these figures and the construction of the Benedict-Roth apparatus makes it easy to express ventilatory volumes on these spirograms, or ones converted to the same dimensions, in terms of the person's estimated vital capacity (Est. V.C.).

Each millimeter of vertical movement of the writing point which traces the Benedict-Roth spirogram represents the movement of 20.7 cc. of gas in or out of the machine. This means that, if the 0.7 is omitted from the calculation, the Est. V.C. of a woman (2,000 cc. per square meter of body surface) can be expressed in terms of this machine as 2,000 divided by 20, or 100 millimeters per square meter of body surface. For a woman the Est. V.C. in millimeters on this

type of spirogram is, therefore, 100 times her square meters of body surface. Any vertical distance on the spirogram can be expressed as per cent of Est. V.C., simply by measuring that distance in millimeters and dividing it by this easily calculated value for Est. V.C.

For a man the conversion of vertical distance on the spirogram to per cent of Est. V.C. is only slightly more difficult. His vital capacity is 2,500 divided by 20, or 125 millimeters per square meter of body surface. For a male subject the Est. V.C. is square meters of body surface times 125. After this calculation has been made, all ventilatory volumes on the spirogram can be converted to per cent of Est. V.C., simply by measuring the vertical length of the spirogram lines in millimeters and dividing by the length of the Est. V.C. in millimeters.

Calculation of total breaths and total inspired volume: In this laboratory the routine procedure in a basal metabolism test is to have two test periods each six minutes long, with additional six-minute trials if the first two give inconsistent figures for oxygen consumption. For convenience the calculations of ventilatory behavior are also based on a six-minute period. The tracings from each metabolism test are inspected and the six-minute period which indicates the lowest oxygen consumption is selected unless there is some obvious defect in that record. After the best six-minute test period is identified, a careful count of the total number of breaths taken and the total length in millimeters of all inspiratory movements of the writing point is made. For this laborious calculation, an adding machine is used. The totals are recorded (figure 2) and a correction factor applied to the figure for inspired volume if a machine other than the Benedict-Roth was used for the test.

Rate of breathing: The average rate of breathing per minute is calculated from the number of breaths taken during the six-minute test period.

Depth of breathing: The average depth of breathing, in millimeters, is determined by dividing the total length of inspiratory movements during the six-minute period by the total number of breaths taken during that time. This figure for average depth of breathing is then converted to per cent of Est. V.C. by dividing by the person's Est. V.C.

Volume of ventilation: The average ventilation in millimeters per minute is determined by dividing the total inspired volume for the six-minute test period by six. This figure can be converted to cc./minute/sq. m. of body surface by multiplying by 20.7 and dividing the product by the person's square meters of body surface. However, a simpler procedure, and one which results in an expression for minute volume of ventilation that is directly related to the person's size and sex, is to divide the figure for average ventilation in millimeters by the Est. V.C. This means that minute volume of ventilation is reduced to the same terms that are employed in measuring other aspects of breathing, namely per cent of Est. V.C. This method is useful for quick clinical inspection of spirograms, since it can be arrived at by multiplying the average depth of breathing in per cent of Est. V.C. by the average rate per minute. That is, a person whose average depth is 10 per cent of Est. V.C. and average rate per minute is 15, has a volume of ventilation per minute equal to 150 per cent of Est. V.C.

Expiratory pause: In most descriptions of normal breathing, a pause at the end of expiration is mentioned. This is based on the idea that expiration is a "passive" movement brought about by elastic recoil of the chest and diaphragm. At first, after the cessation of inspiratory effort the elastic pull is strong and expiration rapid. With return of the thorax and diaphragm toward the resting position, the elastic pull decreases and there is a progressive slowing of the rate of expiration. On a spirogram this decreasing speed of expiration is shown by a gradual rounding of the contour during the latter part of expiration (figure 1a). When there is no expiratory pause, the change from expiration to inspira-

NAME..... AGE..... SEX..... HISTORY #..... PHOTO #.....
CLINICAL DIAGNOSIS..... PULSE..... B.M.R.....
HEIGHT..... WEIGHT..... BODY SURFACE..... EST. V.C.....mm.
BREATHS, total number in 6 min..... INSPIRED VOLUME, total in 6 min.....mm.

RATE/minute: Average..... Highest..... Lowest..... Variation.....
DEPTH: Average.....mm. .....% of Est. V.C.
Largest.....mm. .....% of Est. V.C.
Largest, omitting spikes.....mm. .....% of Est. V.C.
Smallest.....mm. .....% of Est. V.C.
Difference.....% of Est. V.C.

VENTILATION per minute average:.....mm. .....% of Est. V.C.

EXPIRATORY PAUSE: present.....absent.....long.....variable.....
EXPIRATORY POSITION: constant.....moderate var..... marked var.....
Breaths more than 30 of Est. V.C. (.....mm.) from base line:
Number in 6 minutes.....% of 6 minute total.....
SIGHS: Number in.....min. that exceed 2 X Average Depth..... No. per 12 min.....
Number in.....min. that exceed 30% of Est. V.C..... No. per 12 min.....
Sighing increment,.....mm. during 6 min. test.
Inspired Volume (.....mm.) minus sighing increment:.....mm.
Average ventilation/min. without sighs.....mm. .....% of Est. V.C.
Average depth of breathing without sighs.....mm. .....% of Est. V.C.
SWALLOWING: Total number in.....minutes:..... Average:...../min.
INVERTED CONTOUR: Absent..... Variable..... Constant.....
RAPID EXPIRATION: Absent..... Variable..... Constant.....
GENERAL IRREGULARITY: Rate..... Depth..... Base line..... Contours.....

COMMENT:.....

FIG. 2. Form used for tabulation of spirogram data. The methods of anaylsis are described in detail in the text.

tion is represented on the tracing by a sharp point like that normally found at the transition between inspiration and expiration (figure 1b). In some records a long delay is present between successive breaths (figure 1c). For the purposes of this study a long expiratory pause is defined as one in which the adjacent lines of expiration and inspiration, 2 mm. above their lowest point, are separated by a horizontal distance of 2 mm. or more. When this is expressed in terms of time and volume, it means that the final 40 cc. of expiration, the pause itself, and the initial 40 cc. of inspiration on the next breath have consumed more than 3.7 seconds.

Expiratory pause is recorded as present, absent or long only when behavior is consistent throughout the entire tracing. Inconsistent behavior is recorded as variable, with an appropriate secondary designation to show a predominant tendency, if one is obvious, and the range of the variation (for example, *absent* to long).

Sighs: Although sighs are described as deep breaths, there is no single exact definition of a sigh. This is due to the fact that there are two fundamentally different bases for such a definition. Sighs may be defined as breaths twice as deep as the person's average breath, omitting all consideration of the size of this average. They may also be defined as breaths deep in relation to the size and sex of the individual subject, qualifying as sighs on the basis of absolute volume, rather than by exceeding the person's average depth. For individuals whose average depth is moderately large (12.5 to 17.5 per cent of Est. V.C.) the number of sighs is about the same whichever definition is used. When a person has either a small or a very large average depth of breathing, the two definitions give a quite different incidence of sighs.

To study sighs, all large breaths on the whole spirogram are measured. Separate note is made of the number of breaths which exceed two times the person's average depth and the number which exceed 30 per cent of Est. V.C. of the individual. The totals are then expressed as the number of sighs per twelve minutes, since this is the usual length of the tests.

Sighing increment: Suggestions have been made that sighs are intimately related to pulmonary ventilation (10), serving to make up for previously inadequate breathing activity. For this reason it is of interest to calculate the depth and volume of ventilation with the sighing increment omitted to see whether the person's manner of breathing constitutes a reason for his sighing effort. To do this the length of all inspiratory lines which rise in definite spikes above adjacent inspiratory peaks is determined for the six-minute test period. This amount, the sighing increment (figure 3a and 3d), is deducted from the previously calculated total inspired volume. Average depth and minute ventilation are then determined on the basis of this new figure. This procedure is carried out on all records which show significant inspiratory spikes, whether or not these qualify as signs or are of frequent occurrence.

Swallowing: When the glottis is closed, the cessation of air movement is recorded on the spirogram by a horizontal line, since the writing point stops its vertical movement, but the revolving drum maintains its horizontal speed (figure 3e). The presence of a horizontal line is only presumptive evidence that a swallow has been taken, since any abrupt and complete stop in breathing would produce the same result on the tracing. For practical purposes it is necessary to count all definitely horizontal interruptions of the usual breathing contours as swallows. The number for the whole spirogram is determined, and then divided by the number of minutes over which the record extends to give a figure for the average number of swallows per minute.

Individual variation: During the course of a single test individuals may show marked variation in breathing behavior. Some of these alterations can be measured with accuracy, while others are difficult to describe in precise terms.

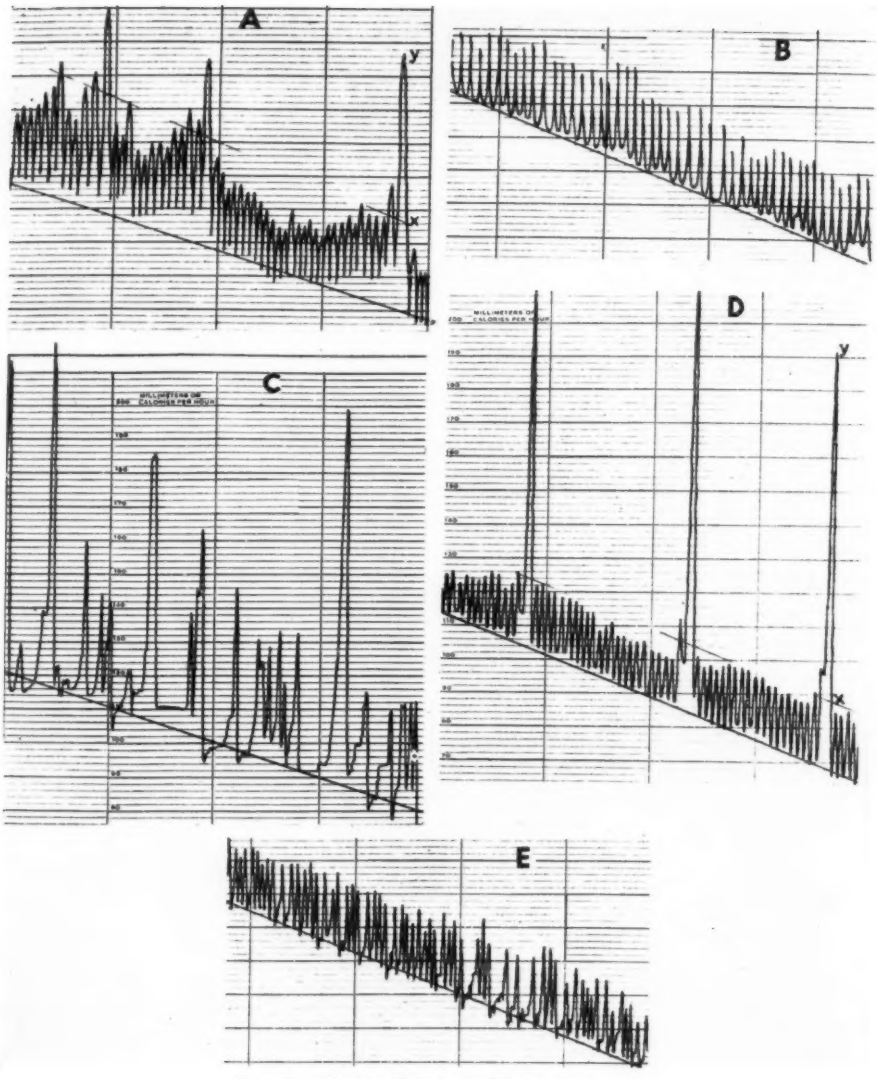

FIG. 3. Illustrative breathing patterns

A. Constant inversion, variable expiratory position, frequent sighs. Sighing increment is the inspiration above broken line x, and is determined by measuring x–y length of all breaths which rise above this line.

B. Rapid expiration, with expiratory line superimposed on that of the previous inspiration for more than one-half its total length.

C. Extreme irregularity of breathing.

D. Even pattern interrupted by large sighs.

E. Horizontal interruptions of breath contours due to closure of glottis during swallowing.

Variation in rate of breathing: Each record is carefully inspected for variations in rate. Count is made for minutes which show rapid breathing and those which

show slow rates. Variation in rate is the numerical difference between the greatest and the smallest number of breaths taken during single minutes, using the entire spirogram, not just the best six-minute period.

Variation in depth of breathing: The term "largest, omitting spikes" refers to the deepest breath in the whole spirogram which does not rise significantly above adjacent inspiratory peaks. "Smallest" breath is applied to the shortest perpendicular movement during what appears on the record to be a free inspiration, not one interrupted by swallowing or grossly different from other small breaths elsewhere in the tracing. When these two, the largest omitting spikes and the smallest, have been measured in millimeters, the difference between them represents the variation in depth which the individual has shown during the test. This figure is divided by the individual's Est. V.C. to give a result in per cent of Est. V.C.

Expiratory position: If breathing is a rhythmic automatic process and ventilatory requirements are stabilized by an adequate rest period, relaxation of the chest in expiration results in a return of lung volume to the same level at the end of successive breaths. Such breathing behavior is shown on a spirogram by an even slope of the expiratory points across the record such that each one just touches a straight line drawn along the base of the tracing (figure 1a and 1b). In many routine tests the patient fails to attain perfect relaxation during expiration. The resulting variations in "functional residual air" are shown by irregularities in the expiratory position (figure 1e and 1f).

Expiratory position is described as constant only when the expiratory extremes consistently reach the base line. The term moderate variation is used to describe deviations about the base line which are minor in degree, transient in duration and not sufficient to cause any difficulty in selecting the proper place to draw the base line. Marked variation is applied to those records in which many breaths fall definitely above or below the general expiratory level, and in which these deviations are sufficiently great and frequent to be an important characteristic of the breathing pattern.

To get a more exact measure of variation in expiratory position, in each spirogram the breaths which fall above or below the base line by a distance as great as, or greater than one-thirtieth of the Est. V.C. of the subject are counted for the six-minute test period. This number is then divided by the total number of breaths taken during that same period to give the per cent of all breaths which deviate to this extent from the base position. It should be pointed out, however, that this is a minimum expression of expiratory variation since it is based on the best six-minute portion of each spirogram.

Inverted contour: Spirograms are inspected for the presence of this pattern, which is characterized by a complete absence of expiratory pause and a definitely rounded contour at the top of inspiration (figure 3a). This breathing behavior, which represents a marked alteration in ventilatory mechanics, is described as constant only when it is maintained throughout the whole spirogram. Under variable are listed all tracings which show this pattern during some part of the test.

Rapid expiration: A breathing pattern which includes a very rapid expiration reveals on the spirogram a superimposition of the line traced during expiration on that of the preceding inspiration (figure 3b). This means that the completion of inspiration and the partial accomplishment of expiration occurred so rapidly that the revolving drum did not have time to move a distance sufficient to give an appreciable separation of the two lines. Rapid expiration is considered present only if the line of expiration is superimposed on that of inspiration for more than one-half of its total length. Records in which all ventilatory movements are rapid because of a consistently fast rate of breathing are not included in this group, which takes in only those patterns which have a normal or long expiratory pause after the rapid movement during the first part of expiration.

General irregularity: Measurement of variations in ventilatory behavior gives only a limited picture of the general character of a spirogram. It is impossible to define in exact terms the quality of even, smooth performance seen in a good record. In such a tracing the rate, depth, expiratory position and breath contours may change, but with transitions so gradual that there is no gross distortion of the pattern. In other spirograms, although the measurable variations in breathing may be moderate, the changes occur abruptly and give a generally uneven appearance to the tracing. It is this latter type of behavior which is recorded under the heading of general irregularity of rate, depth and contour of single breath curves. Under general irregularity of the base line two types of abnormality are included: first, those records in which the expiratory position varies so much during a six-minute test period that it is difficult to select the proper place for the base line; and second, those in which the lines drawn to measure oxygen consumption during successive six-minute test periods show differences in oxygen consumption as great as 5 mm., or 100 cc.

RESULTS

Data presented here are based on a single test from each of the 500 female and 200 male subjects. If a patient had more than one metabolism test, only the one showing the most stable breathing behavior is included.

Standard conditions of temperature and pressure: In calculation of metabolic rate, gas volumes are reduced to standard conditions of temperature and pressure. This is not done in the study of ventilation. Analysis of the factor used in each of the metabolism tests to reduce oxygen volume to 0°C. and 760 mm. pressure shows a maximum range from .866 to .957, with an average of .904 for both men and women. More than 95 per cent of all the 984 records had a correction factor between .880 and .930. It does not seem warranted to inject exactness into the study of a fundamentally unprecise body function. No great error is introduced if it is assumed that these tracings were all taken under atmospheric conditions which would require a correction factor of .904.

Average rate of breathing (table 1): The women show an average rate of breathing from 5.5 to 32.0 per minute. The mean rate is 14.6. One standard deviation below and above this mean gives a range from 11.0 to 18.0, and within these limits are included 72 per cent of the 500 records. There are 13 per cent who

breathe at an average rate of 10.5 or less, and 16 per cent whose rate is 18.5 or more.

Among the men, the average rate ranges from 5.0 to 29.5 per minute. The mean rate is 13.7, and one standard deviation below and above the mean runs from 9.5 to 18.0. Within this range, 73 per cent of the men are included, while 17 per cent of them have an average rate of 9.0 per minute or less, and 10 per cent breathe 18.5 per minute or faster.

Average depth of breathing (table 2): Calculated as an average of all inspiration during a six-minute test period, depth of breathing of the women varies from

TABLE 1

Average rate of breathing, per minute

	500 WOMEN	200 MEN
Range	5.5 to 32	5 to 29.5
Mean	14.6	13.7
Standard deviation	3.7	4.1
Mean ± one standard deviation	11 to 18	9.5 to 18
Per cent of group within this range	72	73
Per cent of group below this range	13	17
Per cent of group above this range	16	10

TABLE 2

Average depth of breathing in per cent of estimated vital capacity

	500 WOMEN	199 MEN*
Range	5.5 to 25.5	5.0 to 24.0
Mean	11.9	11.3
Standard deviation	3.4	3.3
Mean ± one standard deviation	8.5 to 15.0	8.0 to 14.5
Per cent of group within this range	77	78
Per cent of group below this range	9	8
Per cent of group above this range	14	14

* One man with an average depth of 42.5 per cent of Est. V.C. was omitted from these calculations.

5.5 to 25.5 per cent of Est. V.C., with a mean of 11.9 per cent. One standard deviation below and above this mean gives a range from 8.5 to 15.0 per cent of Est. V.C. Within these limits 77 per cent of the 500 women are included. A depth of 8.0 per cent of Est. V.C. or less is shown by 9 per cent of the women, while an average depth of 15.5 per cent of Est. V.C. or more is maintained by 14.0 per cent of the group.

Average depth of breathing among the men ranges from 5.0 to 24.0 per cent of Est. V.C., with one individual maintaining an average depth of 42.5 per cent. If this one subject is omitted from the calculation, the mean average depth is 11.3 per cent. One standard deviation below and above this mean gives a range

from 8.0 to 14.5 per cent of Est. V.C., with 78 per cent of the men included within these limits. Only 8 per cent of the men show an average depth of 7.5 per cent of Est. V.C. or less, while 14 per cent of them have an average depth of 15.0 per cent of Est. V.C. or more.

Average volume of ventilation (table 3): The average volume of ventilation per minute ranges among the women from 100 to 400 per cent of Est. V.C., with a mean of 173 per cent. One standard deviation below and above this mean gives a range from 130 to 215 per cent of Est. V.C., and 77 per cent of the 500 females are within these limits. An average ventilation per minute of 125 per cent of Est. V.C. or less is shown by 10 per cent of the women, while an average of 220 per cent or more is present in 13 per cent of the group.

Among the men the average volume of ventilation per minute varies from 92 to 284 per cent of Est. V.C. The mean is 152 per cent and one standard deviation below and above this mean gives a range from 116 to 188 per cent of Est. V.C., with 77 per cent of the men included within these limits. There are 10 per cent of the men whose average ventilation per minute is 115 per cent of Est.

TABLE 3

Average volume of ventilation in per cent of estimated vital capacity per minute

	500 WOMEN	200 MEN
Range	100 to 400	92 to 284
Mean	173	152
Standard deviation	42	36
Mean ± one standard deviation	130 to 215	116 to 188
Per cent of group within this range	77	77
Per cent of group below this range	10	10
Per cent of group above this range	13	13

V.C. or less and 13 per cent whose average intake per minute is more than 190 per cent of Est. V.C.

Expiratory pause (table 4): The observed behavior in respect to expiratory pause is recorded under four general headings. In the first group are those tracings which show expiratory pause, including: (*a*) consistent expiratory pause, (*b*) predominant tendency to pause, (*c*) long pause and (*d*) variation from normal to long pause. The second group includes those records with a consistently absent pause or a predominent tendency to omit the pause. The third group is made up of those which show a variability in behavior, such that no predominant tendency can be made out. The fourth group consists of those with a maximum variation in behavior, that is, from no pause to a long pause in the course of the same test. The figures grouped in this way show that only 27 per cent of the women and 17 per cent of the men had a consistent, or predominant tendency toward, expiratory pause during these routine metabolism tests. It is also evident that 25 per cent of the women and 34 per cent of the men show no sign of expiratory pause at any time during the whole spirogram.

Sighs (table 5): The incidence of sighs in the routine metabolism records is

shown for each of the two different definitions of sighs. The data indicate that the men sigh somewhat less frequently than the women. Four or more two-times-average-depth sighs per twelve minutes are found in 25 per cent of the women and 20 per cent of the men. If 30 per cent of Est. V.C. is used as the criterion, 4 or more sighs per twelve minutes are present in 17 per cent of the women's records and in 11 per cent of the men's.

TABLE 4

Per cent occurrence of expiratory pause

	500 WOMEN	200 MEN
Expiratory pause present		
Consistently present	9.4	4
Predominantly present	14.2	10
Consistently long pause	.2	1
Normal to long pause	3.2	2
Total	27	17
Expiratory pause absent		
Consistently absent	25	34
Predominantly absent	16	18
Total	41	52
Variation normal to absent	20	23
Variation long to absent	12	8

TABLE 5

Per cent incidence of sighs

	SIGHS 2 × AVERAGE DEPTH		SIGHS 30 PER CENT OF EST. V.C.	
	500 Women	200 Men	500 Women	200 Men
No sighs	35	44	48	59
1 to 3 per twelve minutes	40	36	35	30
4 to 6 per twelve minutes	16	13	9	6
7 or more per twelve minutes	9	7	8	5

Depth of breathing with sighing increment omitted (table 6): When each subject's average depth of breathing is calculated after sighing increment has been deducted from the total inspired volume, the women show a variation from 5.5 to 25.5 per cent of Est. V.C., with a mean of 11.5 per cent. One standard deviation below and above this mean gives a range from 8.5 to 14.5 per cent of Est. V.C., and 78 per cent of the group is included within these limits. Of the 500 subjects, 10 per cent show on this basis an average depth of 8.0 per cent of Est. V.C. or less, while 12 per cent have an average of 150 per cent of Est. V.C. or more.

The 128 women with frequent two-times-average-depth sighs have an average depth varying from 5.5 to 19.0 per cent of Est. V.C., with a mean of 11.1 per cent. There are 73 per cent of these women whose average depth after deduction of sighing increment falls between 8.5 and 14.5 per cent of Est. V.C., while 16 per cent of them have a depth of 8.0 per cent or less and 11 per cent still have an average depth of 150 per cent of Est. V.C. or more.

The 87 women who in twelve minutes have 4 or more sighs as large as 30 per cent of their Est. V.C. show a mean depth of 13.2 per cent of Est. V.C. after

TABLE 6

Average depth of breathing with sighing increment omitted, in per cent of estimated vital capacity

	500 WOMEN	128 WOMEN WITH FREQUENT 2 × AVERAGE DEPTH SIGHS	87 WOMEN WITH FREQUENT SIGHS 30 PERCENT OF EST. V.C.
Range	5.5 to 25.5	5.5 to 19.0	6.5 to 25.5
Mean	11.5	11.1	13.2
Standard deviation	3.1	2.7	4.0
Mean ± one standard deviation	8.5 to 14.5		
Per cent of group from 8.5 to 14.5	78	73	63
Per cent of group 8.0 or less	10	16	8
Per cent of group 15.0 or more	12	11	29

TABLE 7

Average volume of ventilation with sighing increment omitted, in per cent of estimated vital capacity per minute

	500 WOMEN	128 WOMEN WITH FREQUENT 2 × AVERAGE DEPTH SIGHS	87 WOMEN WITH FREQUENT SIGHS 30 PER CENT OF EST. V.C.
Range	75 to 400	75 to 300	75 to 400
Mean	168	163	175
Standard deviation	40	40	50
Mean ± one standard deviation	130 to 210		
Per cent of group from 130 to 210	76	71	64
Per cent of group 125 or less	12	16	15
Per cent of group 215 or more	12	13	21

deduction of the sighing increment. Only 8 per cent of them have an average depth as small as 8 per cent of Est. V.C., while 29 per cent of them maintain an average depth as great or greater than 15.0 per cent of Est. V.C., even after the sighing increment is deducted.

Volume of ventilation with sighing increment omitted (table 7): After the sighing increment is deducted, the average volume of ventilation per minute of the 500 females ranges from 75 to 400 per cent of Est. V.C., with a mean of 168 per cent. One standard deviation below and above this mean gives a range from 130 to 210 per cent of Est. V.C., and 76 per cent of the group are included within these

limits. There are 12 per cent whose average ventilation per minute is 125 per cent or less and 12 per cent whose intake is 215 per cent of Est. V.C. or more.

The 128 women with 4 or more two-times-average-depth sighs per twelve minutes show a ventilation per minute after deduction of the sighing increment varying from 75 per cent to 300 per cent of Est. V.C., with a mean of 163 per cent. Of these 128 subjects, 16 per cent have an average ventilation per minute equal to or less than 125 per cent of Est. V.C., while 13 per cent maintain an average intake of 215 per cent or more.

Among the 87 women with frequent sighs as great as 30 per cent of Est. V.C. there is a range of ventilation per minute from 75 to 400 per cent of Est. V.C., with a mean of 175 per cent. Of this group, 15 per cent show an average ventilation of 125 per cent or less, while 21 per cent of them maintain an average of 215 per cent or more, even after the sighing increment is deducted.

TABLE 8

Per cent incidence of swallowing

	500 WOMEN	200 MEN
Less than 0.5 per minute	42	55
0.5 to 0.9 per minute	29	23
1.0 per minute, or more	29	22

TABLE 9

Individual variation in rate of breathing during single tests, in per cent

	500 WOMEN	200 MEN
3 per minute, or less	44	36
4 or 5 per minute	34	41
6 per minute, or more	22	23

The figures for ventilatory behavior of men with frequent sighs are not presented in detail because of the small number of subjects. In general, these men show the same variations in behavior as those seen in the women who sigh often.

Swallowing (table 8): The horizontal interruptions of normal breathing contours in the routine basal metabolism spirograms indicate that 42 per cent of the women and 55 per cent of the men swallow less than once every two minutes and that 71 per cent and 78 per cent, respectively, swallow less than once per minute. The men swallow less frequently than the women. One female subject swallowed at the rate of 5.7 times per minute throughout her test.

Individual variation in rate of breathing (table 9): Study of the individual variation in rate of breathing during the performance of a single metabolism test shows that 44 per cent of the women and 36 per cent of the men had a variation of 3 per minute or less. Variation of 6 per minute or more appeared in the tracings of 22 per cent of the women and of 23 per cent of the men.

Individual variation in depth of breathing (table 10): Analysis of the variation in depth shown by each individual during the course of a single tracing indicates that the men have a definitely greater variability than the women. Of the women 36 per cent, and of the men only 23 per cent have a variation in depth of less than 7.5 per cent of Est. V.C. There are 31 per cent of the women and 40 per cent of the men who show a variation in depth greater than 10 per cent of their estimated normal vital capacity.

Variation in expiratory position (table 11): The qualitative description of expiratory position under the headings constant, moderate variation and marked

TABLE 10

Individual variation in depth of breathing during single tests, in per cent

	500 WOMEN	200 MEN
Less than 7.5 per cent of Est. V.C.	36	23
7.5 per cent to 10.0 per cent of Est. V.C.	33	37
More than 10.0 per cent of Est. V.C.	31	40

TABLE 11

Expiratory position, in per cent

	500 WOMEN	200 MEN
Constant	9	1
Moderate variation	68	58
Marked variation	23	41

TABLE 12

Variation in expiratory position during best six-minute period, in per cent

PER CENT OF BREATHS ONE/THIRTIETH OF EST. V.C., OR MORE, FROM BASE LINE	500 WOMEN	200 MEN
0	39	42
1 to 5	38	34
6 to 10	11	12
11 or more	12	12

variation is arrived at after inspection of the whole spirogram. The figures show that a constant expiratory position during a routine metabolism test is rare. Marked variation during some part of the test is a frequent occurrence, especially in men. Variation of the expiratory position in terms of the number of breaths which fail to come as close to the base line as one-thirtieth of Est. V.C., is calculated from the best six-minute test period. The data (table 12) indicate that three-fourths of the patients were able to produce one six-minute tracing in which no more than 5 per cent of the breaths deviated to this degree from the base line.

Inverted contour (table 13): The incidence of this breathing pattern is not significantly different between the male and female groups, since about one-third of each show this type of ventilatory behavior at some time during the test.

Rapid expiration (table 14): The figures indicate that this type of breathing is rare. Only 7 per cent of the women and 6 per cent of the men exhibit this pattern even transiently.

General irregularity of pattern (table 15): Precise figures for general irregularities in breathing pattern are presented with hesitation because of the impossi-

TABLE 13

Per cent incidence of inverted pattern

	500 WOMEN	200 MEN
Absent	67	62
Variable	30	33
Constant	3	5

TABLE 14

Per cent incidence of rapid expiration

	500 WOMEN	200 MEN
Absent	93	94
Variable	6	6
Constant	1	0

TABLE 15

Per cent incidence of general irregularities in breathing pattern

	500 WOMEN	200 MEN
No cross iregularity	36	30
Irregularity present	64	70
Type of irregularity:		
Rate	30	27
Depth	33	46
Base line	34	46
Contours	24	19

bility of developing accurate criteria for classification of individual records. However, even a casual inspection of basal metabolism tracings shows that regular breathing throughout the test is not a common occurrence. In the present series 36 per cent of the women and 30 per cent of the men are classed as having no gross irregularity in their breathing behavior. Unevenness is present in more than one phase of ventilation in 38 per cent of the women's records and in 41 per cent of the men's.

DISCUSSION

Objection may be raised to any study of recorded breathing patterns. The patient's knowledge that the test is based on his breathing performance focuses attention on that function and interferes with its normally automatic rhythm. The apparatus used for the recording necessarily introduces chemical and physical factors tending to distort breathing behavior. In spite of these objections, spirographic patterns have significance if they are viewed as an expression of the individual's response to a set of standard conditions. The individual pattern is important when it deviates from the average behavior of persons subjected to the same procedure.

From this point of view, it is not surprising that the tracings taken on a single patient during a series of tests may show a variety of breathing abnormalities. The alterations in breathing recorded during repeated tests vary according to the conditions of the moment and the patient's response to them. However, many patients undergoing repeated tests reproduce on successive tracings an almost identical breathing pattern, even though the interval between tests may be several years (figure 4). Such reproduction occurs most often when the initial behavior is stable and even, but is also seen in records showing very abnormal patterns.

Although it is theoretically probable that average rate, depth and volume of breathing are dependent on individual body structure and neuro-muscular constitution, information is lacking for useful correlations between these mechanical aspects of ventilatory behavior on the one hand and body build and non-respiratory functions on the other. These problems are of immediate clinical interest in relation to anaesthetic and respiratory depressant drugs, breathing response to chest and upper abdominal wounds and maintenance of venous blood flow when the efficiency of the "respiratory pump" is impaired (11).

In spite of the deficiency in knowledge of underlying mechanisms, there is one aspect of breathing pattern which justifies present attention. This is the fact that in recorded breathing patterns one sees revealed graphic evidences of conflict between two fundamental biological processes. On the one side is the rhythm produced by the respiratory needs of the body acting through chemical and physical channels and expressed by the autonomic nervous system. On the other side are the stigmata of voluntary or subconscious interference with this mechanism, ventilatory alterations not related to respiratory needs of the body, but produced by higher brain centres in response to purely emotional or nervous stimuli.

These ventilatory, but not truly respiratory, portions of breathing behavior are of many types. Because breathing is so easily affected by cerebral controls, these variations in spirograms must be interpreted with caution. But frequent sighs, marked lability of ventilatory mechanics, gross distortion of breath contours and inability to maintain an even expiratory position are signs of instability important to the physician who is interested in the constitution of the individual patient. These breathing patterns are comparable in their significance to the instabilities which are frequently observed in heart rate, blood pressure, peripheral blood flow and intestinal motility.

This lack of correlation between basic respiratory needs of the body and the breathing behavior imposed by cerebral influence over the autonomic mechanism, lies behind one important clinical problem, that of hyperventilation (12).

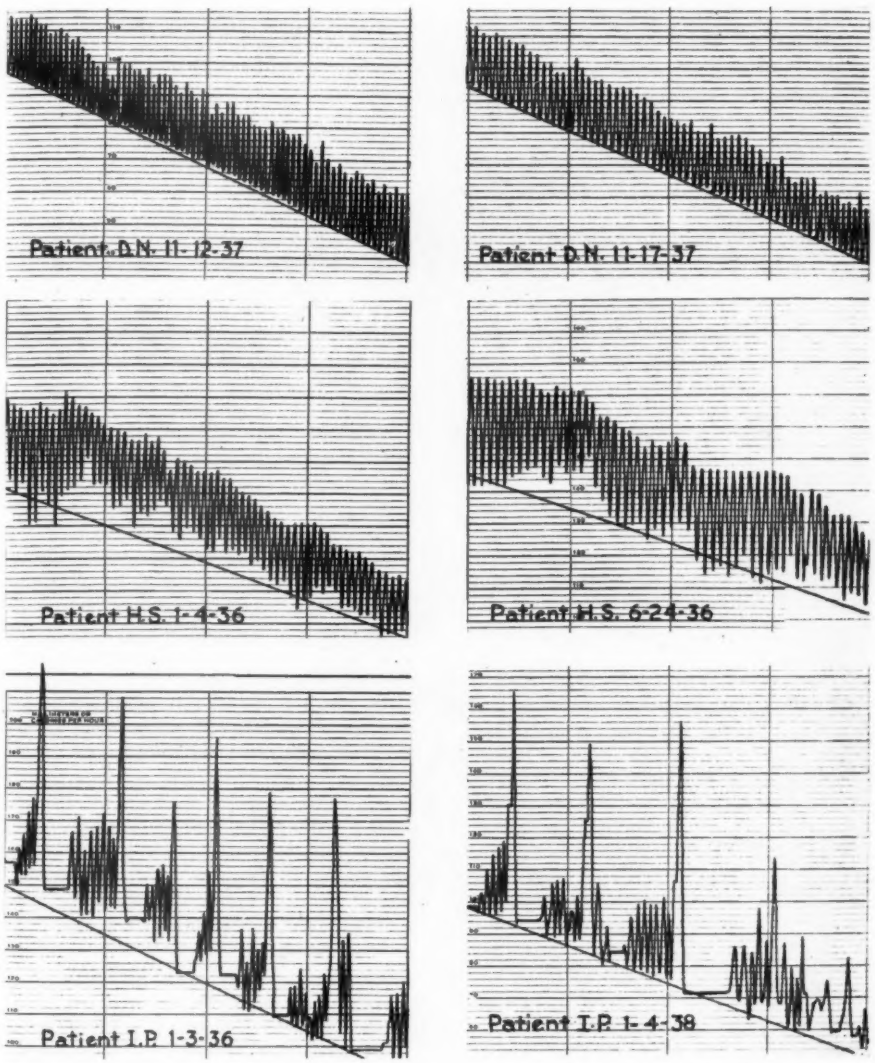

FIG. 4. Reproduction of breathing pattern. Very similar ventilatory behavior shown by these patients during repeated tests. The interval between the tests was D.N. five days, H.S. five months, and I.P. two years.

The natural reaction of any individual who senses some interference with air intake is to fight for more breath voluntarily and with muscular effort gauged to the supposed seriousness of the situation. When the sense of insufficient air intake arises from some normal stimulus, such as increased use of oxygen

during physical exertion, the breathing effort serves a useful purpose. If on the other hand the sense of inadequate ventilation arises from a source other than the respiratory needs of the body, the voluntary breathing effort serves only to remove carbon dioxide in excessive amounts, setting the stage for alkalosis and its sometimes serious clinical manifestations. Common situations in which this nonrespiratory stimulus to overventilation may appear are the substernal pressure of coronary insufficiency, the left chest discomfort of patients with cardiovascular neurosis and the uncomfortable thoracic sensation following a sigh which, though deep, has not attained a sufficient inspiratory peak to give satisfactory muscular relaxation at the end of expiration.

The untrained subject when fighting for his breath concentrates his effort on an increase in rate and depth of inspiration. This leads to progressive difficulty in attaining satisfactory emptying of the lungs during expiration, since little time is left for this part of the respiratory cycle. As the volume of residual air in the lungs increases, there is an inevitable reduction in the amount of air which can be taken into the lungs even with a maximal muscular effort. This gives the patient with neurotic dyspnoea a perfectly sound basis for his characteristic complaint, "inability to get a satisfactorily deep breath" (13). His dyspnoea is increased by the reduction in available ventilatory capacity, the muscle soreness in the chest is aggravated by the excessive strain he imposes on the respiratory muscles (14), yet he cannot increase the volume of air he inspires during a single breath unless he reduces the volume of residual air by allowing adequate expiration.

In the spirogram, the evidences of this kind of breathing stand out, not only in the finding of a large volume of ventilation, but also in the presence of marked and sustained elevations of the expiratory position. These are often accompanied by disappearance of the expiratory pause and an increase in rate of breathing. It is probable that a patient who reacts this way to the minor interference with breathing which occurs during a metabolism test will react in the same direction, and perhaps more vigorously, if a persistent sensation of tightness develops in his chest without any obvious reason.

The figures which have been presented for the ventilatory behavior of women who sigh frequently show that there is little basis for the idea that these large breaths are connected with any respiratory need of the body. Among the sighers there is a wide range of ventilatory behavior even after the sighing increment has been deducted, with only a moderate emphasis on shallow depth among those with two-times-average-depth sighs. On the other hand, when sighs are defined as breaths as great or greater than 30 per cent of the person's Est.V.C. the subjects with frequent sighs are found to have a large average depth of breathing and a large minute volume of ventilation even after the sighing increment is deducted. From a clinical point of view, the appearance of many sighs on a spirogram indicates some kind of emotional or neuro-muscular tension during the recording (15). The chief respiratory problem they suggest is not hypoventilation anoxaemia, but hyperventilation alkalosis, especially if they

are very frequent or superimposed on an already large volume of air intake (16).

No discussion of breathing patterns can be complete without mention of the use of these patterns in the closed circuit graphic method for calculation of basal metabolic rate. The elaborate tables, the slide rules and the multiplications and divisions carried out to two or three decimal places in the estimation of oxygen consumption make many persons forget that the basis for the whole test is the slope of the line drawn along the expiratory points of the patient's spirogram. If the breathing performance of the patient during the test is uneven, particularly if there are alterations in the volume of air in the lungs at the end of expiration, there is no possible way to derive from the record an accurate measurement of basal metabolic rate. Sound clinical practice demands that the physician familiarize himself with what constitutes a satisfactory breathing pattern and refuse to accept any report of basal metabolism unless it is based on a spirogram which is adequate for that calculation (17).

As an initial step in defining some of the characteristics of an average spirogram, the following figures are given. They are based on the performance of an unselected group of patients sent to the laboratory by their physicians, and cannot therefore be assumed to be the same as the breathing patterns which would be found in a study of a series of "normal" individuals.

Average rate: Women 11 to 18 per minute
Men 9.5 to 18 per minute
Average depth: Women 8.5 to 15.0 per cent of Est. V.C.
Men 8.0 to 14.5 per cent of Est. V.C.
Average ventilation: Women 130 to 215 per cent of Est. V.C. per minute
Men 115 to 190 per cent of Est. V.C. per minute
Sighs: Less than 4 per twelve minutes
Swallows: Less than 1 per minute
Variation in rate: 5 per minute, or less
Variation in depth: Equal to or less than 10 per cent of Est. V.C.
Variation in expiratory position: 5 per cent of breaths, or less, miss base line by distance as great as one-thirtieth of Est. V.C.
Inverted pattern: Absent
Rapid expiration: Absent

Since only 27 per cent of the women and 17 per cent of the men show a consistent pause at the end of expiration, such a pause cannot be included as average behavior, even though it is a sign of mental relaxation and an important element in the normal operation of respiratory muscles (18).

SUMMARY

1. Methods are presented for making detailed analysis of breathing behavior recorded during routine basal metabolism tests.

2. Results of such analysis are reported for spirograms of 500 female and 200 male patients.

3. Figures for the average breathing behavior of this group are given.

4. The relation of breathing behavior to some clinical problems is discussed.

5. The dangers involved in uncritical use of spirograms for calculation of metabolic rate are pointed out.

6. It is suggested that analysis of breathing pattern has a place in the study of the physiological and emotional constitution of individual patients.

SUMARIO

1. Los métodos aquí descritos permiten realizar análisis pormenorizados del comportamiento de la respiración según se registra durante la comprobación del metabolismo basal.

2. Preséntase el resultado de dichos análisis para los espirogramas de 200 enfermos varones y 500 mujeres.

3. Preséntanse también las cifras medias para el comportamiento de la respiración en ese grupo.

4. Discútese la relación del comportamiento de la respiración con algunos problemas clínicos.

5. Señálase el peligro que encierra el empleo indiscreto de los espirogramas para calcular el coeficiente metabólico.

6. Indícase que el análisis del comportamiento respiratorio tiene su puesto en el estudio de la constitución fisiológica y afectiva de enfermos dados.

The author's thanks are due to Dr. George Draper and Dr. D. W. Richards, Jr., for helpful suggestions and criticism, and to Dr. C. W. Dupertuis for statistical analysis of the material. Mrs. W. E. Caughey and Mrs. C. L. Holland gave valuable technical assistance.

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NUTRITIONAL STUDIES IN TUBERCULOSIS[1,2]

I. Prothrombin Deficiency and Vitamin K

JASON E. FARBER AND D. K. MILLER

The specific action of vitamin K in the prevention and treatment of bleeding, due to prothrombin deficiency, has naturally led to its use in haemoptysis in tuberculosis. Recent reports (1, 2, 3) indicate that a lowered plasma prothrombin occurs in approximately 30 per cent of tuberculous patients and that the hypoprothrombinaemia reaches its lowest level in patients with pulmonary haemorrhage. Levy (1) determined the prothrombin time of 60 patients and administered synthetic vitamin K (menadione) to 14 patients with moderate haemoptysis. Scheely (2) noted that the prothrombin concentration decreased in proportion to the degree of toxaemia of the tuberculous. In his series of 106 patients, 51 patients showed a significant prothrombin deficiency. He treated 4 haemoptoic patients with menadione. Bauer (4) found a definite hypoprothrombinaemia in a group of 30 patients with a history of repeated haemoptysis. Twelve of these patients responded to vitamin K therapy. In another group of 10 patients with haemoptysis the prothrombin concentration was within normal limits. He concluded that, although a reduction in prothrombin does not constitute the cause of haemoptysis, the duration and intensity of the bleeding may be influenced by treatment with vitamin K in cases in which the prothrombin concentration is considerably diminished. All these investigators agreed that vitamin K is a helpful factor in the control of haemoptysis. On the other hand, Kaplan (5) and Harrell and Ray (6) reported that vitamin K was of no value in the control of haemoptysis. In view of the limited scope and the controversial nature of these studies and because of the wider implications of hypoprothrombinaemia as part of a nutritional deficiency, it was believed justifiable to make a more complete and thorough investigation of the subject.

METHODS OF STUDY AND MATERIAL

The method of Quick (7) was used to determine the prothrombin clotting time. This is measured in seconds and when compared with the prothrombin time of a normal control gives a reliable estimation of the plasma prothrombin level. The greater the elevation of the prothrombin time over the normal control, the greater is the prothrombin deficiency. In using the Quick method it is not permissible to express the clotting time in percentage, since a linear relationship does not exist between prothrombin concentration and the plasma clotting time (8). A delay of three seconds or longer in prothrombin clotting time appears to indicate a significant prothrombin deficiency, although it is not the level at which bleeding occurs. For this reason all results have been classified into two groups, those with a prothrombin time of more than three seconds and those with a prothrombin time of less than three seconds.

[1] From the Edward J. Meyer Memorial Hospital and the University of Buffalo School of Medicine, Buffalo, New York.

[2] This study was aided by the Buffalo Tuberculosis Association.

More than 700 determinations of the prothrombin time were made on some 400 patients with pulmonary tuberculosis. Approximately 100 control tests were performed on normal persons. In this series, there were 116 patients with streaked sputum and haemoptysis, including those with massive haemoptysis. These patients were all treated parenterally with menadione[3] (2-methyl-1-4 naphthoquinone), except for a small group which were used as controls. The patients were divided into two groups, the nonbleeding patients and those with haemoptysis or streaked sputum. The effect of vitamin K and of sodium phenobarbital on the prothrombin time was also studied.

TABLE 1

Prothrombin time of nonbleeding and bleeding patients

SECONDS*	NONBLEEDING		BLEEDING	
	Number	Per cent	Number	Per cent
0	137	31	24	21
1	81	19	14	12
2	73	17	16	14
3	53	12	17	14
4	36	8	15	13
5	16	4	12	10
6	13	3	4	3.5
7	7	1	4	3.5
Over 7	21	5	10	9
Total	437		116	
Percentage of cases with prothrombin time three seconds or longer..		33		53

* Seconds prolongation over the normal control.

RESULTS OF PRESENT STUDY

Prothrombin time in nonbleeding and bleeding patients: As shown in table 1, 437 prothrombin determinations were made on nonbleeding patients and 116 on bleeding and streaking patients. One hundred forty-six, or 33 per cent, of the nonbleeding and sixty-two, or 53 per cent, of the bleeding patients had a prolongation of the prothrombin time of three seconds or longer. It can also be seen in table 1 that the plasma prothrombin reaches a considerably lower level in bleeding than in nonbleeding tuberculous patients. Nine per cent of the bleeding patients had a prolongation of the prothrombin time of more than seven seconds as compared with 5 per cent of the nonbleeding patients. There was not always a correlation between the amount of bleeding and the prothrombin time. Occasionally a massive haemoptysis was observed in a patient on whom a normal prothrombin time was found. For example, a patient, J. W., under treatment with vitamin K, had a normal prothrobmin time (fifteen seconds, control fifteen

[3] A supply of menadione (hykinone) was obtained through the courtesy of Dr. J. F. Biehn, Abbott Laboratories, Chicago, Illinois.

seconds) on two successive days during which time he suffered haemoptyses, totaling 800 cc. and 400 cc. However, in patients with only slight streaking of the sputum the prothrombin values corresponded roughly with those of nonbleeding patients.

Effect of vitamin K on the prothrombin time: Daily prothrombin determinations were made on a group of 26 patients whose prothrombin time was prolonged and who were receiving vitamin K. Sixteen of the patients were bleeding. In most instances the prothrombin time returned to normal within twenty-four to seventy-two hours. In only a few of the patients with bleeding was the prothrombin time refractory to vitamin K. Although the prothrombin time was restored to normal in most of these cases, the patients often continued haemorrhaging. This was particularly true of patients with large haemoptyses. On

TABLE 2

Prothrombin time of nonbleeders arranged according to clinical status

SECONDS*	FRANKLY ACTIVE PATIENTS		INACTIVE OR QUIESCENT	
	Number	Per cent	Number	Per cent
0	54	19	83	53
1	51	18	30	19
2	50	18	23	14
3	45	16	8	5
4	31	11	5	3
5	13	5	3	2
6	11	4	2	1
7	7	3	0	0
Over 7	17	6	4	3
Total	279		158	
Percentage of cases with prothrombin time three seconds or longer..		44		14

* Seconds prolongation over the normal control.

the other hand, some of the patients with smaller haemoptyses often stopped bleeding abruptly, coincident with the restoration of the prothrombin level. The dose of menadione was 3.2 mg. daily. Larger doses were given in a number of instances but no additional benefit resulted.

To serve as a control for the above experiment, daily prothrombin determinations were made on a group of 9 patients with haemorrhage in whom the prothrombin time was prolonged and who did not receive vitamin K. In contrast to the rapid (twenty-four to seventy-two hours) return to normal observed in patients treated with vitamin K, the prothrombin time of these untreated patients did not return to normal until seven to eight days had elapsed.

Prothrombin time and the clinical status of the patient: A pronounced deficiency of prothrombin, as evidenced by a considerable lengthening of the prothrombin clotting time, was observed frequently in patients dying of tuberculosis. In

view of this, the prothrombin determinations of moderately and far advanced tuberculous patients were arranged according to the clinical status of the patient at the time the test was performed. As seen in table 2, 279 patients were classified as frankly active and 158 patients as inactive or quiescent. Forty-four per cent of the former group and 14 per cent of the latter group had an increase in the prothrombin time of three seconds or longer. It is evident, that the prothrombin deficiency reflects the clinical status of the patient.

Effect of sodium phenobarbital on the prothrombin time: Prothrombin determinations were made on 26 nonbleeding patients receiving sodium phenobarbital. These determinations were done just before and just after the parenteral administration of 0.26 to 0.32 g. of the drug. In only one instance was the prothrombin time increased three seconds, in 5 instances it was increased from one to two seconds and in the remainder it was not increased. It seems apparent that under the conditions of the experiment, the administration of sodium phenobarbital did not influence the prothrombin time.

DISCUSSION

The results of this study, in agreement with those of other investigators (1, 2, 4), show that a prothrombin deficiency occurs in tuberculous patients. This deficiency is more pronounced in bleeding patients. It is difficult to evaluate the significance of the prolonged prothrombin time in bleeding patients in relation to their haemorrhage. It has been shown that a moderate loss of blood does not lower materially the prothrombin concentration of the plasma (9). In further support of this is the group of patients who suffer pulmonary haemorrhage lasting for days and who show little change in the prothrombin time. If this is true, it is highly unlikely that bleeding is the cause of the abnormal prothrombin levels of these patients. In view of the fact that a considerable number of the nonbleeding patients also had a prolonged prothrombin time, it is suggested that the prothrombin deficiency is an indication of the poor nutritional status of such patients and not the result of haemorrhage. It is of interest that the prothrombin deficiency manifests itself especially in sick patients, that is, those with fever and other constitutional symptoms. This corresponds to other manifestations of vitamin deficiency such as of vitamins A (10), C (11), riboflavin and niacin (12). Thus, the deficiency reflects the clinical status of the patient rather than the anatomical extent of the pulmonary involvement.

A fairly large group of patients with a definite hypoprothrombinaemia have been observed for months without the occurrence of a single haemoptysis. It would appear, then, that haemoptysis cannot be predicted from a determination of the plasma prothrombin concentration. On a theoretical basis, one could assume that a patient with a prolonged prothrombin time and in addition an ulcerative pulmonary lesion would be more apt to bleed and to bleed more severely than a patient with a similar lesion and a normal prothrombin level. This study, however, has not shown this to be true.

One conclusion reached by Levy (1), Scheely (2) and others (3) is that vitamin K is effective in stopping pulmonary haemorrhage. If this were so, a remarkable

therapeutic substance would be available in vitamin K. Unfortunately our studies do not bear this out. Since haemoptyses invariably stop spontaneously without any treatment or even in spite of some forms of treatment, it is not possible to prove that a given drug or measure was responsible for terminating the bleeding. This holds similarly for vitamin K. In the present series, many patients with haemoptysis, while under treatment with menadione, continued bleeding for several days and occasionally for many days. It is known that bleeding in tuberculosis is due to ulceration or erosion of a blood vessel by the tuberculous process. There is little need, then, to postulate a deficiency of prothrombin or vitamin K as the cause of the haemoptysis. The next question which arises is whether the duration of bleeding might not be affected by the prothrombin level, particularly since prothrombin is essential for the clotting of blood and since the prothrombin level is restored so rapidly by vitamin K, as shown in the present studies. It was found that although the prothrombin time returned to normal there was no observable effect of vitamin K on the haemorrhage.

Concerning the relationship of prothrombin deficiency to tuberculosis and to haemorrhage, as seen in tuberculosis, the following can be stated with some degree of accuracy. Prothrombin deficiency occurs in about one-third of tuberculous patients and in a somewhat higher percentage of progressive tuberculous patients. The deficiency manifests itself in about 50 per cent of bleeding patients and reaches its lowest levels in this group. It is important to consider this deficiency as a part of a general nutritional deficiency. Synthetic vitamin K is effective in restoring the prothrombin concentration and should be administered for this purpose only. It has not been established that vitamin K stops pulmonary haemorrhage or influences its course.

SUMMARY AND CONCLUSIONS

1. Prothrombin deficiency was found in 33 per cent of the nonbleeding and 53 per cent of bleeding tuberculous patients.

2. Synthetic vitamin K (menadione) restored the plasma prothrombin concentration but had no effect on the haemorrhage.

3. Prothrombin deficiency reflects the clinical status of the patient and is a manifestation of a general nutritional deficiency.

SUMARIO Y CONCLUSIONES

1. Descubrióse deficiencia de protrombina en 33 por ciento de los tuberculosos sin hemoptisis y 53 por ciento de los que tenían hemoptisis.

2. La vitamina K (menadiona) restableció la concentración de protrombina en el plasma, pero no afectó la hemorragia.

3. La deficiencia de protrombina refleja el estado clínico del enfermo y constituye una manifestación de una deficiencia general de la nutrición.

Acknowledgment is made to Miss June Hood and Mr. Ross Imburgio for technical assistance.

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NUTRITIONAL STUDIES IN TUBERCULOSIS[1,2]

II. Niacin (Nicotinic Acid) and Riboflavin Deficiency

JASON E. FARBER AND D. K. MILLER

The study of vitamin nutrition in tuberculosis has been concerned primarily with vitamins A, C and D. Getz (1) and others (2) have shown that avitaminosis A and C is common in tuberculosis. Little, however, has been written on deficiencies of the B-complex in this disease. The present study shows that deficiencies of niacin and riboflavin are common in tuberculosis and that the specific treatment of these deficiencies is of importance in the treatment of the disease.

Jolliffe (3) has divided the nutritional deficiencies into two types, primary and secondary malnutrition. The former is due to an inadequate intake of essential food elements, whereas the latter is produced by certain conditioning factors. These conditioning factors may be physiological, such as physical exertion or pregnancy, or pathological, such as illnesses which increase requirements, impair absorption, utilization and retention, or hasten excretion of essential nutrients. Tuberculosis might easily fit into both categories, since many patients with this disease come from the lower economic strata and subsist on an inadequate diet, while the disease, *per se*, may produce the so-called conditioning deficiency factors.

MATERIAL AND METHODS OF STUDY

Approximately 400 patients were studied in regard to niacin and riboflavin deficiency. Of this group, 100 patients showed definite changes in the tongue, lips or mucous membranes of the mouth. Complete clinical, roentgenological and laboratory studies were made on each of these patients. Attention was directed particularly to the clinical status of the patient rather than to the anatomical extent of the disease process; all patients under discussion had advanced pulmonary tuberculosis. When evidences of glossitis, stomatitis or cheilosis were noted the patient was selected for more careful study. This included gastric analyses, blood counts and serial prints and photographs of the tongue. Detailed descriptions of the tongue, lips and mouth were made.

Tongue prints: These were made as follows (4): the patient was asked to project his tongue which was wiped dry and covered with black ink by means of a stamp pad. Then a stiff white card was applied to the tongue with a rolling motion and quickly removed. The use of the serial tongue prints has proved to be a valuable and objective method for the study of the glossitis. These records serve to identify the type of glossitis as well as changes in the tongue under vitamin therapy.

Clinical evidences of niacin and riboflavin deficiency: Two distinct types of

[1] From the Edward J. Meyer Memorial Hospital and the University of Buffalo School of Medicine, Buffalo, New York.

[2] This study was aided by the Buffalo Tuberculosis Association

stomatitis and glossitis were observed in these patients, one considered to be the result of niacin, the other of riboflavin deficiency. Not infrequently a com-

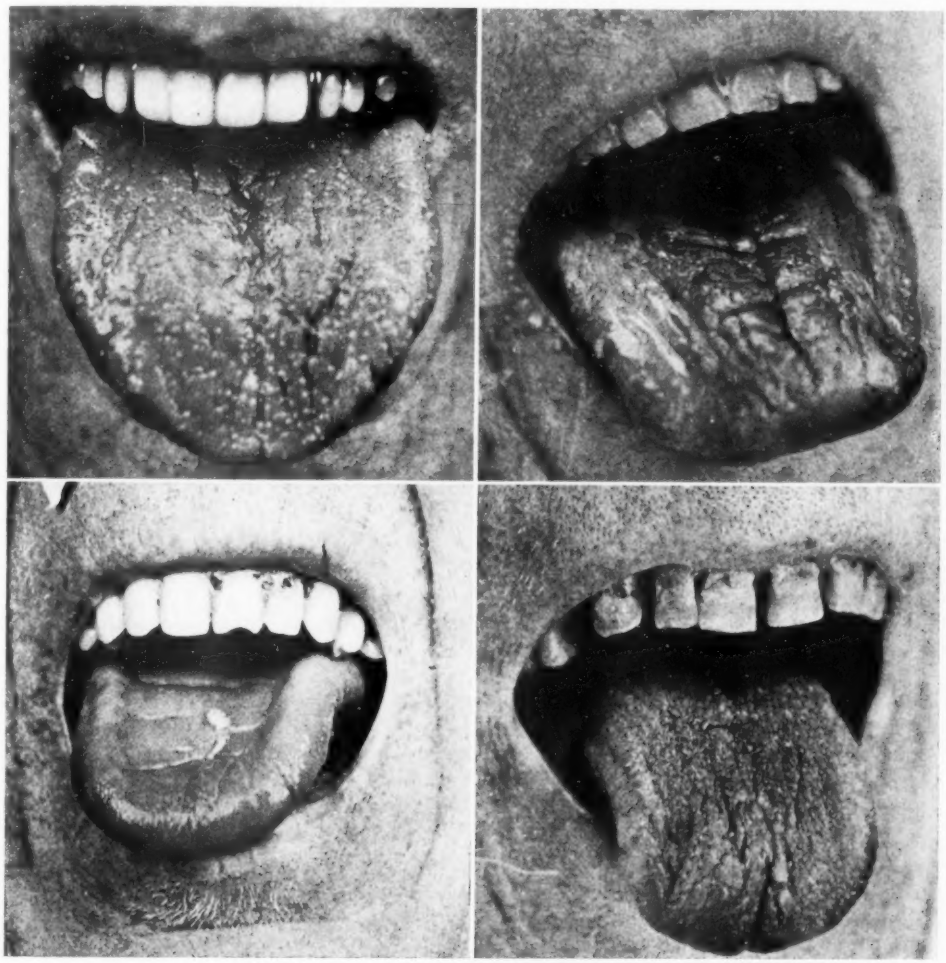


FIG. 1. (Upper left.) Photograph of a niacin deficiency tongue showing hypertrophy of the papillae.

FIG. 2. (Upper right.) Photograph of a niacin deficiency tongue showing hypertrophy of the papillae. This tongue was fiery red.

FIG. 3. (Lower left.) Photograph of a bald atrophic tongue. The papillae returned after niacin therapy.

FIG. 4. (Lower right.) Photograph of the tongue of case 2 showing furrowing and hypertrophy of some of the papillae. The tongue was magenta colored indicating a riboflavin deficiency.

bination of the two deficiencies was seen. The glossitis of niacin deficiency is as follows: at the onset, the tip and lateral margins of the tongue show slight reddening and hyperthrophy (figure 1) or atrophy (figures 2 and 5) of the papillae.

As the condition progresses the tongue becomes swollen, dry and scarlet or beefy red. In the more severe cases all the papillae atrophy, the tongue becomes smooth and shiny and finally the red color disappears leaving a smooth, pale, bald tongue (figures 3 and 6). The response to niacin therapy is remarkable. The tongue returns to normal in the course of days, providing adequate therapy is administered. The mucous membranes of the mouth occasionally show color changes similar to the tongue as well as superficial ulceration.

The riboflavin deficiency tongue is often fissured and of a purplish red or magenta color. It is associated with other evidences of riboflavin deficiency, that is, reddening and transverse fissures at the corners of the mouth, reddening

TABLE 1

*Lesions in 100 patients with niacin and riboflavin deficiency**

	LESIONS	NUMBER OF CASES
Glossitis	Scarlet or beefy red tongue (niacin)	21 pronounced 24 moderate
	Purplish red or magenta tongue (riboflavin)	9 pronounced 11 moderate
	Atrophy of papillae	17 pronounced 12 moderate
	Hypertrophy of papillae	3
Stomatitis	Ulcerations of mucous membranes (niacin)	7
	Cheilosis, angular stomatitis (riboflavin)	10
	Combined niacin and riboflavin deficiency	8

* Since combinations of lesions were often present some cases are included under more than one heading.

and superficial denudation of the lips (cheilosis), seborrhoeic dermatitis of the nose, ears and outer canthi of the eyes.

OBSERVATIONS

Frequency of niacin and riboflavin deficiency in tuberculosis: The 100 patients with niacin and riboflavin deficiency represent about 25 per cent of the patients examined. Borderline or subclinical deficiencies were not included in this series. Therefore, the frequency of these deficiencies may be greater than 25 per cent. There seemed to be a direct relationship between the incidence of nutritional deficiency and the clinical status of the patient. As a matter of fact, more than 75 per cent of the acutely ill patients showed some evidence of avitaminosis. As seen in table 1, there were more instances of glossitis than of lesions of the mucous membranes. Forty-five of the patients were considered to have chiefly

a niacin deficiency as evidenced by glossitis; 21 of these were of pronounced and 24 were of moderate degree. Twenty of the patients were considered to have chiefly a riboflavin deficiency as evidenced by glossitis; 9 of these were pronounced and 11 were of moderate degree. When atrophy or hypertrophy of the papillae was seen, without having observed the preceding clinical picture it was difficult to evaluate the vitamin aetiology. The impression was obtained that atrophy of the papillae is more likely to follow niacin than riboflavin deficiency. The mucous membrane lesions in patients showing a niacin deficiency occurred in at least 7 instances. These were characterized by oedema, marked redness and superficial ulcerations. In 10 patients with riboflavin deficiency there was angular stomatitis. In 8 patients the lesions suggested a combination of both riboflavin and niacin deficiency.

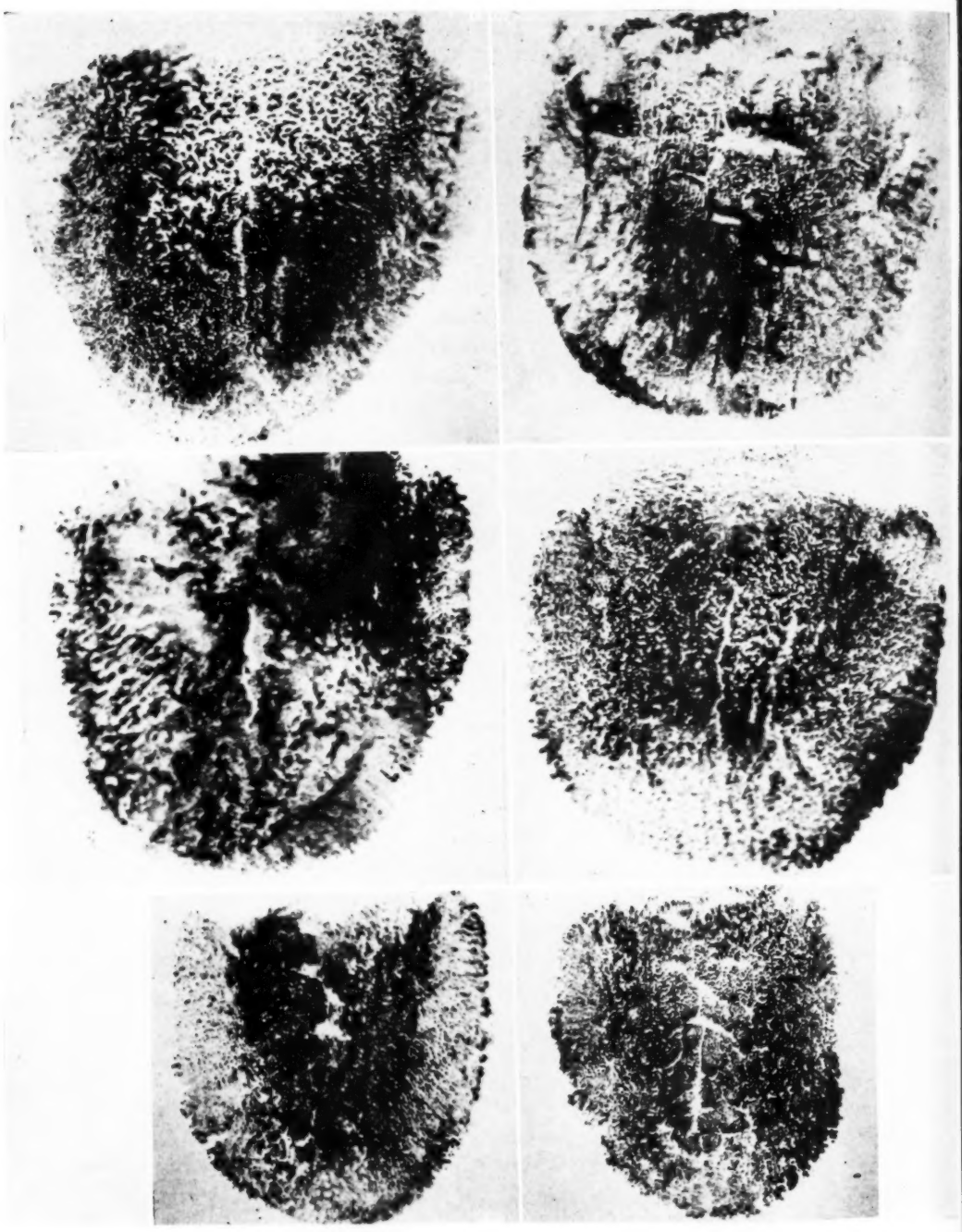
Age, sex and race: The ages of the 100 patients ranged between eighteen and eighty-one, the mean age being forty-nine. There were 93 male and 7 female patients. Eighty-nine were white and 11 were colored patients.

Nutritional history: Many of the male patients came from homes for transients and from rooming houses, a few were from the County Infirmary. On admission they were often in a deplorable state of health and nutrition. A primary malnutrition was undoubtedly chronically present in these cases but the tuberculosis added the conditioned deficiency factors. The female patients had chiefly a conditioned deficiency due to their tuberculosis. An appreciable number of the patients had clinical evidences of intestinal tuberculosis, amyloidosis and empyema.

Treatment: All patients were maintained on bed-rest and given a high caloric, well balanced diet. Therapeutic measures were instituted whenever indicated for the various tuberculous and nontuberculous complications. Fifty patients received vitamin therapy. This consisted of 3 ounces of Brewer's yeast, 150 mg. of niacin and 6 mg. of riboflavin daily. Some patients required considerably larger doses. It was occasionally necessary to administer 300 to 600 mg. of niacin daily to certain acutely ill patients, much of this being given parenterally. The total number of cases treated is obviously too small for statistical analysis. It can be stated, however, that improvement in the local lesions under vitamin therapy occurred in approximately 85 per cent of cases, whereas only about 10 per cent in the untreated group showed improvement. As a rule, improvement in the lesions of the tongue and lips was rapid. In a typical case the earliest sign of improvement was a reduction in the swelling and a return of the moistness of the tongue. In most instances redness then gradually disappeared. In the cases with atrophic glossitis the reappearance of the papillae was rapid (figures 7 and 8). Among those patients who showed only temporary improvement were several with tuberculous meningitis and amyloidosis, complications which inevitably led to death.

Two cases of the present series were selected for detailed presentation.

Case 1: Pulmonary tuberculosis, acute glossitis, stomatitis and cheilosis, mental symptoms; treatment with niacin, thiamine, riboflavin and yeast; recovery. G. Z., aged forty-three,



FIGS. 5–10

with known pulmonary tuberculosis became acutely ill with pain in the chest and fever ten days prior to admission. On examination he appeared to be a poorly nourished white male, acutely ill, dehydrated, disoriented, irrational, hallucinatory and in need of restraint. He had frequent emesis and occasional convulsions. His cough was hacking and productive. His temperature was 100°F., the pulse rate was 130. The lips were ulcerated and crusted, the angles of his mouth were fissured and the tongue was swollen, dry and bright beefy red. Subdued bronchovesicular breath sounds and numerous râles were heard over the upper half of both lungs. The heart was rapid but no bruits were heard. There was no paralysis of the extremities but the patellar reflexes were absent. The reflexes of the upper extremities were hyperactive.

The patient remained acutely ill for several weeks. His temperature ranged between 97° and 102.4°F. The pulse rate was correspondingly elevated. His tongue was sore and painful on motion. The dehydration was treated and the patient was given daily 300 mg. of niacin orally and parenterally, 30 mg. of thiamine, 90 g. of Brewer's yeast and 6 mg. of riboflavin. The patient soon showed remarkable improvement. The ulcerated lips began to heal and he began to eat. The mental symptoms cleared rapidly, he became rational and oriented. His cough lessened somewhat. He became semiambulatory. The tongue gradually returned to normal size and became moist. The redness slowly disappeared but the papillae did not return for many weeks. Tongue prints were made at intervals (figures 9 and 10).

The sputum was positive for tubercle bacilli and negative for pneumococci. A roentgenogram of the chest showed a fibrotic tuberculous infiltration in the upper lobes of both lungs. The Wassermann and Kahn tests were negative. The urine was normal. The blood count was not remarkable. The blood sugar was 70 mg. per 100 cc. Spinal fluid examination revealed questionable dynamics, it was clear and colorless, had 2 leucocytes per cu. mm. and no globulin. Gastric analysis before the administration of specific therapy showed a reduced volume and no free hydrochloric acid in all four samples, even after the injection of histamine. Fifteen days later the gastric juice was of good volume and showed 37 degrees of free hydrochloric acid after an alcohol test meal. The plasma prothrombin time was nineteen seconds, the control fifteen seconds.

Case 2: Advanced pulmonary tuberculosis, pronounced toxaemic symptoms, avitaminosis with glossitis; improvement with yeast, niacin and riboflavin. F. W., aged sixty-two, related a history of productive cough, streaked sputum, night sweats, anorexia and loss of 25 lbs. in weight. On examination the patient appeared emaciated and acutely ill. His face was flushed and the skin was hot and moist. The pupils were slightly dilated, the sclerae were pearly white. His tongue was dry, swollen, somewhat fissured and fiery red. Altered breath sounds and showers of râles were heard over both lungs. His heart was rapid. The blood pressure was 130 mm. Hg systolic and 68 mm. diastolic. The abdomen and extremities were normal.

FIG. 5. (Upper Left.) Tongue print showing atrophy of some of the papillae.

FIG. 6. (Upper right.) Tongue print of the same patient showing complete atrophy of the papillae.

FIG. 7. (Middle left.) Tongue print of a patient showing atrophy of the papillae.

FIG. 8. (Middle right.) Tongue print of the same patient after two weeks of niacin therapy.

FIG. 9. (Lower left.) Tongue print of case 1 soon after the onset of vitamin therapy.

FIG. 10. (Lower right.) Tongue print of the same patient during the course of vitamin therapy showing the return of many papillae.

His temperature ranged between 98° and 101.6°F., the pulse rate was 80 to 100. The patient sweated profusely day and night. He was kept on strict bed-rest, given a high caloric diet and 150 mg. of niacin daily. A week later his tongue appeared a purplish red or magenta color (figure 4). He was then given yeast and riboflavin. After several weeks his tongue returned to normal color, size and moistness.

His sputum was positive. A roentgenogram of the chest revealed an extensive bilateral, fibrocavitary pulmonary tuberculosis. The blood count was within normal limits. The urine was normal. The Wassermann test was negative. The prothrombin time was nineteen seconds, the control fourteen seconds. Gastric analysis showed good volume and free hydrochloric acid.

DISCUSSION

In contrast to the considerable interest manifested in vitamins A and C in tuberculosis there has been a paucity of reports on deficiencies of the vitamins of the B-complex in this disease. Three observations on this subject have appeared in the literature, two (5, 6) concerned with thiamine and one (7) concerned with the B-complex. In the present series niacin and riboflavin deficiencies were seen in about 25 per cent of the patients examined. This is not unexpected in view of the nature and socio-economic implications of tuberculosis. Conditioning deficiency factors are present in tuberculosis as in other diseases, such as pneumonias, ulcerative colitis, alcoholism and gastrointestinal malignancy in which these deficiencies have been reported (8, 9). These conditioning factors are well illustrated in the present study. Fever and the accompanying elevated metabolism of active tuberculosis increase the body requirements of vitamins. In patients with enterocolitis, causing hypermotility of the bowel and diarrhoea, there is impaired retention and absorption and rapid excretion of the essential nutrients. In patients with amyloidosis, particularly of the liver, there might be interference with the utilization of the vitamins. The loss of proteins in renal amyloidosis is also serious. In patients with prolonged haemoptysis the standard treatment which included only liquids in the diet has produced manifestations of avitaminosis in some of the reported cases. Patients with tuberculous meningitis and other severe forms of the disease usually eat poorly and, with increased requirements incident to fever, infection, etc., develop deficiency syndromes. Some of the most pronounced niacin-deficiency tongues were in this type of patient. Four patients with empyema also developed evidences of nutritional deficiency. Numerous nontuberculous complications which either unbalance the tuberculosis or influence the patient in other adverse ways were frequently seen. In short, a patient with tuberculosis who suffers any one of manyfold infections or disease conditions, such as diabetes mellitus, peptic ulcer, infections of the urinary tract, etc., may develop a nutritional deficiency.

Relationship of nutrition to the severity of the tuberculosis: The state of nutrition of a tuberculous person can usually be correlated with the severity of the disease process. Certainly the very sick and dying patient is in a poor state of nutrition. He is emaciated, his muscle tone is poor and his strength is gone.

Careful examination often will demonstrate evidence of multiple deficiencies, of vitamins A, C and K (10) as reported in the literature, and of niacin and riboflavin as observed in the present study. As a matter of fact, most of the patients with glossitis of the severest type were encountered among our sickest and dying patients. Thus it was observed that, as a rule, the sicker the patient, the more frequent and the more severe was the nutritional deficiency. On the other hand, there was a group of patients whose poor clinical status was all out of proportion to the anatomical extent of their tuberculosis. A great many of these were homeless men and alcoholics. In these cases, treatment, when directed specifically to the nutrition, resulted in a rapid clinical improvement.

Therapeutic considerations: The treatment of a conditioned vitamin deficiency must take into consideration the treatment of the conditioning factor as well as the specific deficiency. Hence, the usual treatment of tuberculosis is first of all essential in these cases. The diet should be liberal and of high vitamin content. Adjuvants such as codliver oil and tomato juice are also valuable. In addition to all this, specific therapy for the nutritional deficiency is further indicated. With evidence of niacin deficiency a minimum dose of 150 mg. should be given daily in divided doses. In addition, at least one ounce of Brewer's yeast should be given three times a day to cover sub-clinical deficiencies of other portions of the B-complex which might be present. In acutely ill patients with severe manifestations of niacin deficiency, treatment should be prompt and energetic. In these cases, in addition to a diet of at least 4,000 calories, 500 mg. of niacin should be given daily in divided doses, orally or parenterally. In some instances even larger doses may occasionally be necessary.

In patients with ariboflavinosis, yeast should be given with 6 to 15 mg. of riboflavin daily. There were several patients receiving niacin and riboflavin for glossitis whose tongues did not improve until large amounts of yeast were also given. This suggests that perhaps other members of the B-complex may participate in the deficiency syndrome. Thus, in most instances niacin and riboflavin and yeast were administered to each patient. In agreement with Jeghers (11) the tongue may be considered an index of nutritional deficiency. Once seen, the swollen, sore, beefy red tongue cannot be forgotten. Furthermore, the response to specific therapy is equally dramatic as it is diagnostic. Serial tongue prints were of great value in objectively following the course of these deficiencies and their response to therapy.

SUMMARY AND CONCLUSIONS

Twenty-five per cent of 400 patients with tuberculosis showed definite evidence of niacin and riboflavin deficiency. The principle types of lesions encountered were glossitis, cheilosis and mucous membrane lesions. These lesions respond rapidly to a proper diet and specific vitamin therapy. The sicker the patient the more frequent and the more severe is the nutritional deficiency. This study emphasizes the fundamental importance of nutrition in tuberculosis.

SUMARIO Y CONCLUSIONES

Un 25 por ciento de 400 tuberculosos revelaron signos bien definidos de deficiencia de niacina y riboflavina, consistiendo las lesiones principales en glositis, queilosis y lesiones de las mucosas que responden rápidamente a una alimentación apropiada y vitaminoterapia específica. Mientras más enfermo está el sujeto, más frecuente y más intensa es la deficiencia nutritiva. Este estudio recalca la importancia fundamental de la nutrición en la tuberculosis.

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FLUORESCENCE MICROSCOPY OF MYCOBACTERIUM TUBERCULOSIS[1]

A Study of Basic Factors

CLARENCE F. GRAHAM

Fluorescence microscopy for the identification of *Mycobacterium tuberculosis* has not been accepted with much enthusiasm in this country, and the author has had many unfavorable reports from persons who have attempted its application. Without further preliminaries, it may be said that the method is worthless if not properly applied. Heretofore no comprehensive study has been made of light sources, requisite excitation wave-band, optical system, filters or stains, so that the procedure is still largely empirical. The present investigation was made to determine these basic factors as the foundation on which to evaluate the method.

In principle, fluorescence microscopy depends for its success on the ability of certain dyes to reradiate visible light when illuminated by light of a shorter wavelength. By using suitable filters that absorb the direct illuminating radiation but transmit the differently colored light due to fluorescence, organisms bearing the dye are made visible.

When the technique is applied to the identification of *M. tuberculosis*, the yellow fluorescent dye auramine is employed, since it readily stains acid-fast organisms which then resist decolorization with acid. Violet light is used for illumination and a yellow disc in the microscope ocular filters out the violet light of the background and allows fluorescent organisms to stand out on a darker field.

EXPERIMENTAL APPARATUS

A spectrograph was constructed with a reflecting replica grating of 25,000 lines to the inch and a focal length of 83 cm. This was mounted to rotate on a vertical axis through the centre of the grating and tilted slightly forward to throw the spectrum onto a front surface aluminized mirror set at 45 degrees to the optical axis so that the focused spectrum was received on a film in a 5 x 7 inch camera at right angles to the optical box. The length of the spectrum from the mercury line at 2967 Å to the extreme visible red was about 32 cm. The razor blade slit was varied from .031 to .85 mm. according to the condition of the exposures. It is to be noted that by this arrangement, no light absorption is effected except on the reflections from the grating and mirror, and for the qualitative result desired these may be safely neglected.

In order to avoid the necessity of calibrating the spectrograph, each film was given its own calibration lines by a short exposure to the arc spectrum of a large quartz lamp (Hanovia). For making band spectrum photographs, a 500 watt

[1] From the Hermann M. Biggs Memorial Hospital, Division of Tuberculosis, NewYork State Department of Health, Ithaca, New York.

120 volt G.E. projection bulb was used as the light source. Although this lamp has a glass envelop that effects a certain amount of absorption in the ultraviolet, the spectral distribution is nevertheless satisfactory for the experiments.

Eastman Tri-X panchromatic film Type C, 5 x 7 inch was used and showed excellent sensitivity from the ultraviolet well into the red.

Auramine-O used for the studies was made by the National Aniline and Chemical Co., Inc., and was of lots No. 9690 and 9966.

The thioflavin was obtained through the courtesy of the General Aniline Works, Rensselaer, New York.

THE WAVE-BAND OF EXCITATION FOR THE FLUORESCENCE OF AURAMINE-O AND THIOFLAVIN

Fluorescence may be caused by any one of the wave-lengths present in a certain region of the spectrum, the particular region required depending on the nature of the fluorescent substance. Within this spectral interval, there is usually a rather confined group of wave-lengths that produces the maximum fluorescent response, and the illuminating source should be designed to concentrate the greatest possible amount of its light into this region. A determination of the excitation band of auramine is essential if the best results are to be obtained with this dye as a staining agent for fluorescence microscopy. There seems to be an impression, particularly among the German workers, that the excitation maxima for auramine and thioflavin lie in the ultraviolet and that a mercury quartz lamp is required in order to make the stained organisms show up brightly. Such is not the case, however, as the results of the present study prove.

The experimental method has not previously been described so far as the author knows.

Two unexposed photographic films were cleared in hypo, and dyed yellow, one with saturated aqueous auramine solution and the other with saturated aqueous picric acid solution. Visually, the films after drying were quite similar in color. The two dyed films were fastened together along their edges. A photographic film was then exposed to the mercury arc lines in the spectrograph, following which the dyed films were placed over it and then an exposure was made to the band spectrum of a 500 watt projection bulb for thirty minutes. The procedure was repeated with another photographic film with the pair of dyed films reversed so that the light passed through them in opposite order. Thus two spectrograms were obtained, the one when the auramine was toward the light and the picric acid filter toward the photographic film, and the second when the picric acid filter was toward the light and the auramine toward the photographic film. The procedure somewhat corresponds to the use of an intensifying screen in X-ray photography. When the raw spectrum falls first on the auramine film, fluorescence is excited in the auramine by a certain range of wave-lengths, and the yellow light of the fluorescence passes through the picric acid filter and leaves its imprint on the photographic film over the range of the activating light, with the wave-lengths identified by the mercury arc lines. When the spectrum falls first on the picric acid film, only yellow light reaches the auramine and no fluores-

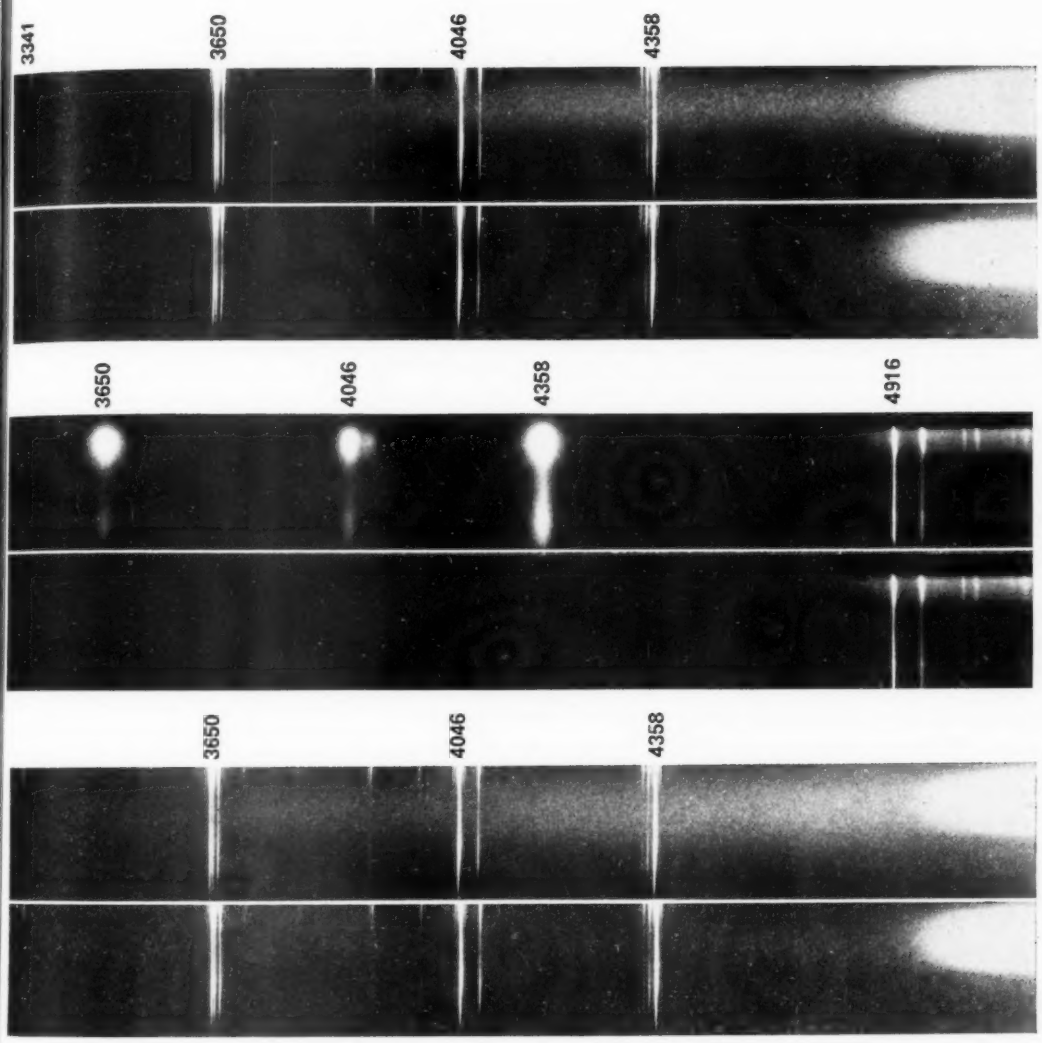

FIG. 1. (Top) Excitation wave-band of the fluorescence of auramine-O

Upper spectrogram: raw spectrum of 500 watt projection bulb thrown on auramine-dyed film resting on picric-acid-dyed film, resting in turn on the photographic film. Fluorescence excited by wave-lengths from 3400 to 4700 Å; broad maximum in vicinity of 4500 Å.

Lower spectrogram: same spectrum thrown on picric-acid-dyed film resting on auramine-dyed film, resting in turn on the photographic film. No fluorescence excited when light passes first through the yellow picric acid film.

FIG. 2. (Center) Excitation of the fluorescence of auramine-O by the line spectrum of the mercury arc

Upper spectrogram: spectrum of quartz mercury arc thrown on auramine-dyed film resting on picric-acid-dyed film, resting in turn on the photographic film. Fluorescence excited by all the high intensity lines, but maximum at 4358 Å.

Lower spectrogram: same spectrum thrown on picric-acid-dyed film resting on auramine-dyed film, resting in turn on the photographic film. No fluorescence excited.

FIG. 3. (Bottom) Excitation band of the fluorescence of thioflavin

Upper spectrogram: raw spectrum of 500 watt projection bulb thrown on thioflavin-dyed film resting on picric-acid-dyed film, resting in turn on the photographic film. Fluorescence excited by wave-lengths from 3400 to 4700 Å; broad maximum in vicinity of 4500 Å.

Lower spectrogram: same spectrum thrown on picric-acid-dyed film resting on thioflavin-dyed film, resting in turn on the photographic film. No fluorescence.

cence results. Since the two films together are dense enough to filter out practically all the blue and ultraviolet light of the spectrum, the photographic film is not darkened when the light reaches it first through the picric filter and then through the auramine.

Figure 1 shows two spectrograms obtained in this fashion. The upper is made with the auramine toward the light and the picric acid filter toward the photographic film and indicates that fluorescence has been excited in the auramine by wave-lengths from 4700 Å to 3400 Å, with a broad maximum at about 4500 Å.

The experiment was repeated to determine which of the mercury lines excited the most intense fluorescence. The spectrograms are shown in figure 2 and indicate by the size of the flare about the lines that the visible high-intensity line at 4358 Å is the most effective. This result is in accord with the determination of the excitation band maximum at 4500 Å, since 4358 is the nearest high-intensity line to the band maximum. The line 3650, often used in ultraviolet photomicrography, is quite evidently less effective for fluorescence microscopy than the visible line 4358. It should be remembered that the light did not pass through any glass whatever in the making of these spectrograms, so that glass absorption of the ultraviolet was not a factor.

The determination was repeated with thioflavin since this dye has been recommended for fluorescence microscopy of the tubercle bacillus, and it was thought worth while to compare its excitation band and brilliancy of fluorescence with those of auramine. Figure 3 shows the spectrograms obtained with thioflavin by the same method used with auramine. It will be noticed that fluorescence ceases slightly to the long-way side of the mercury line at 3650 Å, and in general is rather less brilliant over the whole range than that of auramine.

This single observation applies only to the particular lot of thioflavin used and may not be generally true, but it corroborates the author's observation that the tubercle bacillus fluoresces less brilliantly with thioflavin than with auramine. Adding to this the fact that thioflavin is not generally stocked by chemical supply houses, while auramine is relatively cheap and easily obtained, it would seem that there is no advantage in substituting thioflavin for auramine as the stain for fluorescence microscopy of tubercle bacilli.

WAVE-BAND OF THE FLUORESCENT LIGHT OF AURAMINE

This determination was simple after the extremely low intensity of the fluorescent light was appreciated. The slit was set at 0.85 mm. and the light of a 500 watt projection bulb focused on it by a pair of 15 cm. diameter plano-convex lenses so that the condensed filament image on the slit covered an area about 2 cm. in diameter, so intense as to set fire to black paper in a few seconds. A flat-sided jar of ammoniacal copper sulfate solution served as a violet filter; the auramine in a thick paste with a little gelatin was coated on a glass plate and a Corning Glass Works Noviol Shade D, #3384 polished two inch square was used as the yellow filter. The auramine film and the #3384 filter were fastened together and held in a pocket on one side of the jar containing the cuproammonium solution, and precautions were taken to prevent light from reaching the slit except through the violet solution and the auramine-filter combination.

With all three components in position, the system was practically opaque even to strong light. An electric fan cooled the cuproammonium jar throughout the

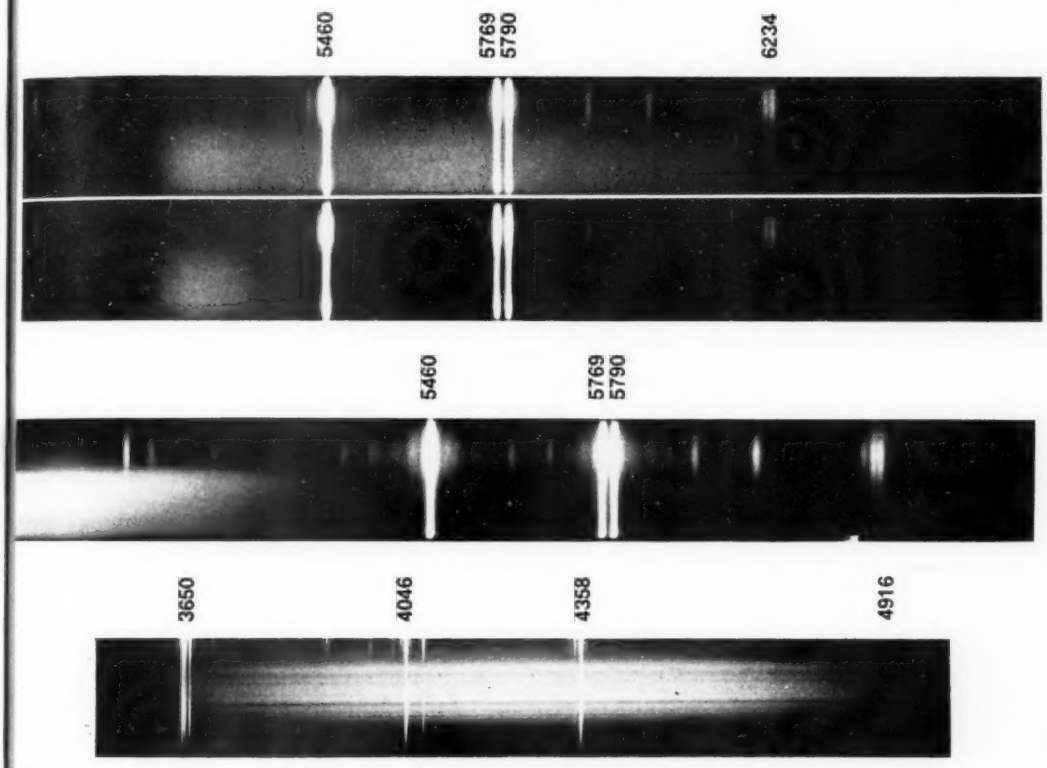

FIG. 4. (Top) Wave-band of fluorescent light of auramine-O in dry gelatin

Upper spectrogram: strongly condensed light of 500 watt projection bulb passed through cuproammonium solution, then through auramine-gelatin film, then through Corning Glass yellow filter #3384 to the 0.85 mm slit; twelve hours' exposure.

Lower spectrogram: same condensed light passed through cuproammonium solution, then through C. G. yellow filter #3384, then through auramine-gelatin film to the slit; twelve hours' exposure.

The transmission band at the left end of the lower spectrogram must be subtracted from the upper spectrogram to give the fluorescence band which extends from 5400 to about 6500 Å

FIG. 5. (Center) Determination of fluorescent efficiency of auramine-O

Strongly condensed light of 500 watt projection bulb passed through cuproammonium solution as in figure 4, but auramine-gelatin film and yellow filter #3384 omitted; exposure five seconds.

FIG. 6. (Bottom) Transmission band of ammoniacal copper sulfate solution (cuproammonium) as used for fluorescence microscopy

exposures and the level of the filter solution was maintained by replenishing with ammonia water.

Even with the inordinate slit width and strongly condensed light, exposures of twelve hours were required to produce satisfactory spectrograms of the fluores-

cence band. Figure 4 shows spectrograms obtained with the auramine film toward the light and the yellow filter toward the slit (upper) and with the yellow filter toward the light and the auramine film toward the slit (lower). The principle is the same as that used to obtain the excitation band. When the exciting light, after passing through the cuproammonium solution falls on the auramine film, yellow fluorescence is excited in the auramine, transmitted by the Noviol filter with about 15 per cent loss of intensity, and is sorted out by the spectrograph into its wave-lengths. When the exciting light falls first on the Noviol filter, no fluorescence is excited in the auramine and the wave-band of the fluorescence is absent, but a small band of transmission in the blue-green region is registered and must be subtracted from the other spectrogram to give the true fluorescence band. In this determination, only one variable was altered, that is, the order of the auramine film and the yellow filter relative to the light and the slit.

The wave-band of the fluorescent light of dry auramine in gelatin was found to extend from 5400 to 6500 Å.

EFFICIENCY OF AURAMINE AS A FLUORESCENT DYE

The fluorescent efficiency of a dye is defined as the ratio between the amount of light which it emits as fluorescence and the amount which it absorbs. The ratio of the exposure times required to produce the fluorescence wave-band print and an equally dense print with the activating light alone would be a rough determination of the fluorescent efficiency of auramine. When all the light transmitted by the violet filter was absorbed by the auramine film and the yellow glass, enough fluorescent light was produced in twelve hours to give the photographic effect shown in figure 4. The violet light alone produced an equally dense trace in five seconds, as shown in figure 5. Assuming equal absorption of violet light in the auramine film and the yellow glass (though probably the auramine absorbed at least 80 per cent of the initial violet light) the fluorescent efficiency of auramine in gelatin would be, very roughly, the ratio between five seconds and six hours, or not over 0.02 per cent.

This is in accord with the results of visual observation and may be compared with the fluorescent efficiency of uranin (fluorescein-sodium) between 70 and 80 per cent. Probably the only reason auramine can be used at all for detection of the tubercle bacillus by fluorescence is because the fluorescent light lies in the region of greatest retinal sensitivity. Curiously, the extreme inefficiency of auramine as a fluorescent dye offers the best hope for improvement of the whole technique by discovery of some dye with high fluorescent efficiency and the same acid-fastness in the tubercle bacillus. The organism can be stained by long exposure to a saturated aqueous solution of auramine and rhodamine-B, and fluoresces red in the violet light passed by cuproammonium solution. Only a few preliminary experiments have been made in this direction, but they indicate that further study might be repaid.

FILTERS TRANSMITTING THE EXCITATION BAND

The only commercial glass filter so far discovered which transmits the excitation band with good practical efficiency is the Corning Glass Works Violet, #5113. Unfortunately, this glass has so high a coefficient of expansion that it will not withstand the fierce heat of the 500 watt projection bulb even when it is mounted almost in contact with the front plano-convex condensing lens so as to spread the heat over the greatest possible surface. The Corning Glass Works states that no annealing process will obviate this difficulty which is inherent in the glass. Attempts to cement the cracked filter to a glass backing and fill the cracks with paint have not been successful.

The chief advantage of a glass filter is convenience in mounting on the lamp. For actual use with the microscope, it has been found preferable to use a flat-sided museum jar filled with a strong solution of ammoniacal copper sulfate, made by adding strong ammonia water in excess to a saturated aqueous solution of copper sulfate, and adjusting the density by dilution. When properly adjusted, auramine-stained tubercle bacilli under the microscope stand out in strongest contrast to the background, depending upon the preference of the observer. Some workers prefer a darker field and less brilliant organisms, and others a lighter field and brighter fluorescence. It should be realized that a black field will not be obtained with the strong condensed light of a 500 watt projection bulb, since all organic matter on the slide will fluoresce somewhat, usually with a vague, pale, grayish-green color. A black field means simply low intensity of illumination, and introduces the difficulty that the plane of the slide is hard to locate while at the same time the organisms fluoresce faintly, since the fluorescence is proportional to the intensity of the exciting light.

No other violet filter solution has been found that even approaches the cuproammonium in efficiency. All the numerous blue and violet dyes tested for the purpose have shown considerable transmission of red light, so that they were completely useless. The ideal filter would be a heat-resistant plastic of proper color, but nothing of the sort is obtainable at present.

The transmission band of cuproammonium solution is shown in figure 6 for comparison with the excitation band of auramine.

FILTERS TRANSMITTING THE FLUORESCENCE BAND OF AURAMINE FOR USE IN THE OCULAR

The transmission requirements of the yellow disc are not particularly critical and many types of glass are satisfactory. The ocular disc should not pass too much of the green light that gets through the violet filter in small amount at the end of its transmission band, but, at the same time, it should transmit as much light as possible on the long-wave side of 5200 Å in order to utilize all the faint fluorescent light of the stained bacilli in the spectral region where retinal sensitivity is the greatest, near 5600 Å. Most yellow filters transmit red light freely, so that the long wave part of the fluorescence band takes care of itself.

Corning Glass Works yellow filters, #3384, #3386 and #3484 as the polished discs are all satisfactory, but require cutting and grinding for use inside the ocular. The Spencer Lens Company ocular disc for fluorescence microscopy is efficient; its transmission starts well away from the green so that it gives a slightly orange tone to the organisms. Perhaps the most easily obtainable filter is the Imperial "dark yellow" filter used as a photographic filter and sold as a 25 mm. disc, which can easily be ground down on a carborundum wheel to drop into the ocular. It can be bought at many photographic stores and has excellent transmission characteristics.

LIGHT SOURCES FOR EXCITATION

Naturally the best light source for the excitation of auramine fluorescence would be the one that concentrated the most energy at the peak of the excitation curve, in the region of 4500 Å. Several types of light may be considered.

Arc lamps: An open carbon arc is highly effective, but is impractical because of its rapid consumption of carbons and the difficulty of maintaining proper feed. Impregnation of the carbons with various salts or the use of metallic electrodes can improve the efficiency but will not eliminate the inconveniences.

Mercury lamps: The various forms of high or low pressure mercury lamps give good excitation of fluorescence by the strong lines at 3650, 4046 and especially 4358 Å, but require transformers or reactors, and the light units are usually obtainable only from the makers. Most of these applicances are designed to have the greater part of their output in the ultraviolet, where it is less effective for exciting the fluorescence of auramine.

Spark sources: Spark sources of radiation, using metallic spark points, could easily be designed for high energy output in the desired region of the spectrum, but would require transformers and condensers, would need complete shielding to prevent radio interference, and would have to be operated under license from the Federal Communications Commission. Proper design, however, could produce a light with high emission in the desired region. Spark points of platinum or titanium would emit a number of high intensity lines close to 4500 Å. These sources have not been tested for fear of producing interference with radio communication.

Argon bulbs commercially available are of low power and their spectrum contains few intense lines in the vicinity of 4500 Å.

Hot filament lamps: There remains the class of hot filament lamps, most of them designed for general illumination, but a few specially constructed to produce intense light for projection purposes. Lamps of the latter type are usually "overvoltaged," that is, the filament is shorter in the overall length of its wire than it would be for normal operation at the rated voltage of 115–120 volts. In consequence, the filament is considerably hotter than the usual filament and the emission band spectrum is shifted toward the short wave end of the spectrum, with an accompanying reduction in the life of the filament and more rapid blackening of the glass. Photoflood bulbs are of this type, but do not lend themselves to the purpose of fluorescence microscopy because the glass of the bulb is frosted and the filament cannot be focused in the microscope.

Projection bulbs have a clear glass and a concentrated filament of several coils, so that a fairly uniform filament image can be concentrated in the field of the microscope. They are obtainable with standard screw bases to fit the common socket and are sold at many photographic stores. The cost of operation, on a basis of fifty hours' average life for the 500 watt bulb, is about five cents an hour. They produce almost as much heat as an electric toaster or a flat-iron and will melt soft solder from a lamp housing. Their efficiency is low in the region of the spectrum where high output is desired to excite fluorescence of auramine. In spite of these disadvantages, it is the belief of the author that a 500 watt projection bulb in conjunction with a pair of 4 inch (10 cm.) diameter plano-convex

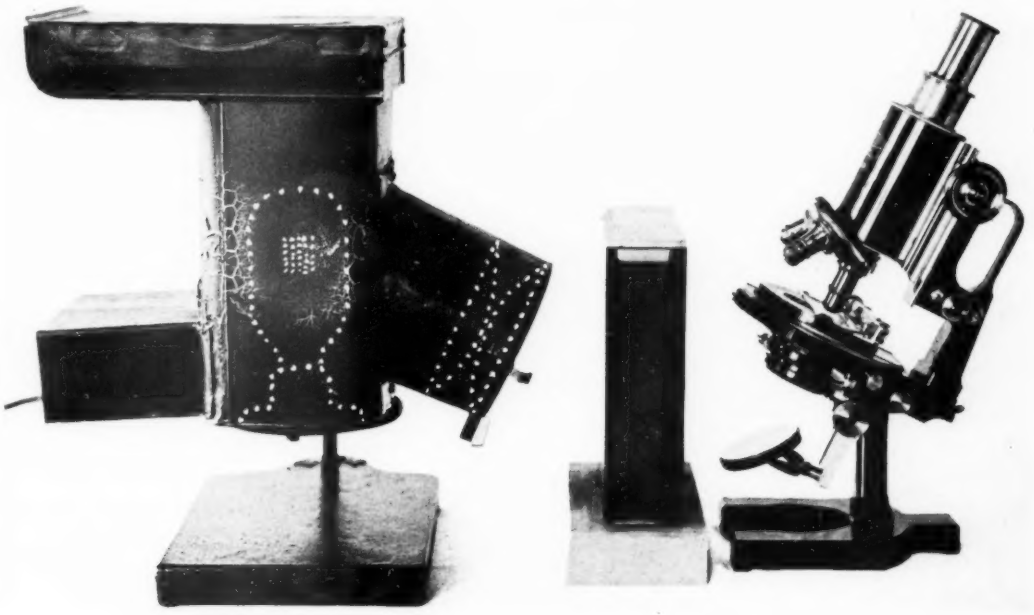

FIG. 7. Improvised illuminator for fluorescence microscopy, using a G.E. 120 volt 500 watt T-20 projection bulb and a pair of 10 cm. diameter plano-convex condensing lenses. The positions of bulb and lenses are indicated by the dotted lines.

condensing lenses is the most practical arrangement that can be improvised under present conditions for fluorescence microscopy with auramine as the staining medium. Much better light sources can probably be expected after the war.

An improvised light assembly is shown in figure 7. This was built from a one-gallon paint can supplemented by sheet metal from another larger can, and carries a G. E. 500 watt 120 volt T-20 projection bulb in a porcelain socket. The beam is condensed by two 4 inch (10 cm.) diameter plano-convex lenses obtained from a photographic supply house, and mounted with the convex sides together. The distance from the convex sides of the lenses to the filament should be such that the beam converges to the microscope mirror at a convenient dis-

tance and nearly covers the mirror. Machine screws should hold the housing together in addition to any solder used. The angle mounting of the lens tube allows the lamp to burn in an upright position and, at the same time, defocuses the filament image in the microscope field so that it is more uniform. The socket is bolted through the base so that it can be turned to set the plane of the filament at about 30° to the optical axis and bring the images of the coils closer together in the microscope field. The housing is bolted to the ring of a ring-stand for adjustment of height.

The light is a considerable improvement over that previously described by the author (1).

Many factors enter into fluorescence microscopy and, if they are understood, some difficulties may be avoided or corrected.

OPTICAL PROPERTIES OF THE LIGHT SYSTEM

The properties of the glass composing the whole optical system have a great effect on the satisfactory working of the method. The light that excites the fluorescence of auramine lies in the visible spectrum and the long-wave ultraviolet and, since colorless optical glass transmits light freely on the long-wave side of 3400 Å, no difficulty would arise if the whole system were made of the best water-clear glass. The least tinge of yellow, however, even below the limit of visibility, greatly reduces the excitation light and cuts down the fluorescence. The condensers of some old-style, cheap microscopes have this defect, and the instruments are useless for fluorescence microscopy. It is possible that the same yellowish coloration might occur in the flat-sided museum jar used for the cuproammonium solution, but any color in this case is usually a slight green and does not interfere with transmission of the activating light.

FLUORESCENCE OF MICROSCOPE OBJECTIVES

A much more serious difficulty is the fluorescence of microscope objectives themselves, often so intense in high power lenses as to obscure the fluorescent bacilli completely. Unfortunately apochromatic fluorite lenses of the highest quality may be the worst offenders, since many fluorite crystals exhibit strong visible fluorescence and even find their way into collections of fluorescent minerals. If the primary search of a stained film is made with a 16 mm. lens, and the morphology of any fluorescent organisms is confirmed with a 4 mm. lens, strong fluorescence in the high power lens may make the examination entirely futile. There is no way to eliminate this disturbing effect, which is inherent in the glass itself as well as in the gum damar used to cement the lens components together. A number of 4 mm. lenses were tested in actual use and all fluoresced so strongly as to be worthless for practical purposes.

COMBINATION DARK-FIELD AND FLUORESCENCE MICROSCOPY

A combination of dark-field and fluorescence microscopy cuts down the lens fluorescence by eliminating the axial pencil of light, and thus results in better

contrast between organisms and field, but requires the additional step of making immersion contact between the top of the condenser and the bottom of the slide. If this method is adopted, however, methyl salicylate (oil of wintergreen) may, and preferably should, be used as the immersion oil, since it does not fluoresce to any extent. This oil is easily wiped off the slide or evaporates in a few days, if forgotten. The combination method makes it necessary to set aside a microscope for fluorescence microscopy alone or to go to the trouble of changing condensers for each examination. In the author's limited experience with a high-grade dark-field condenser, the results were excellent, but equally dark fields were obtained by the method of counterstaining described later.

FLUORESCENCE OF SLIDES

Faint fluorescence can be detected in the glass slides used for smears, but has not been any impediment in a number of sample slides examined with this possibility in mind. It might conceivably cause trouble with occasional batches of glass and should be considered as a possible cause of an excessively light field.

CHOICE OF OBJECTIVE AND OCULAR

An 8 mm. objective with 10x ocular is entirely practical for primary search and confirmation of the morphology of any fluorescent organisms. The field of an 8 mm. objective has four to five times the ~~area~~ diameter of that given by the usual oil immersion lens, so that a much larger part of a stained film can be searched in the same time for both lenses, or a shorter search with the 8 mm. lens will cover the same area examined by the immersion lens. In direct smears correctly stained with auramine only acid-fast organisms fluoresce to any degree, so that the general flora of the sputum is invisible. This may be a decided objection to the method, since useful leads are often obtained from a Ziehl-Neelsen stained film which reveals all the types of organisms present while emphasizing acid-fast bacilli.

BINOCULAR MICROSCOPE

A binocular microscope is not satisfactory for fluorescence microscopy even with the strong condensed light of the 500 watt bulb, for the multiple prism system reduces the illumination of the field below a practical level.

Microscope mirror: The plane mirror of the microscope is used and should be kept clean and free from dust in order not to reduce the intensity of the excitation light.

STAINING METHOD

Heat-fixed films are stained in the cold for two minutes with a saturated aqueous solution of auramine, freshly made on the day of use. The addition of phenol to the stain is not necessary, and the most brilliant fluorescence is obtained from fresh stain.

The slide is rinsed in running water and decolorized for thirty seconds with Gabbett's methylene blue solution; methylene blue, 2 g., in 100 ml. of 25 per cent

sulfuric acid. It is then rinsed and examined. Longer decolorization may be needed in the case of thick smears. The methylene blue counterstain greatly reduces the fluorescence of organic matter making up the background of the film and the fluorescent organisms appear in better contrast to the field.

Stained films fade quickly in strong light and the sooner they are examined after staining the better will be the results. They can be restained several times after fading.

TRAINING OF THE OBSERVER

Anyone who plans to use the fluorescence method for the identification of the tubercle bacillus should spend some hours examining known positive films stained with auramine and counterstained with Gabbett's methylene blue acid solution. The technique is not automatic and some judgment must be exercised to disregard the faintly fluorescent irregular particles that occur even in the best preparations. Only clear-cut, definitely fluorescent narrow rods should be reported as acid-fast bacilli morphologically resembling *M. tuberculosis*. The amount of technical judgment required is less than that needed for the identification of the diptheria bacillus in routine smears.

DISCUSSION OF THE FLUORESCENCE METHOD

Advantages: (1) The staining technique is simpler than the Ziehl-Neelsen method, since it eliminates heating or prolonged exposure to the stain.

(2) The use of an 8 mm. objective in place of an immersion lens allows the examination of larger film-area in the same time, or the same film-area in shorter time.

(3) Under proper conditions, the examination of films by the fluorescence method involves less eye-strain than the examination of Ziehl-Neelsen preparations.

(4) Slides are examined dry without the nuisance of immersion oil.

Disadvantages: (1) The method requires a special light system which is neither efficient nor cheap in operation.

(2) A liquid filter must be used.

(3) An 8 mm. objective is almost a necessity and may be unobtainable during the war.

(4) Efficient optical equipment is necessary, though not beyond the requirements of any well-provided laboratory.

(5) Examinations must be made in a darkened room.

In the author's opinion, the method is not ready for general use until it has been evaluated by test under the most favorable conditions that can be afforded by the average laboratory. The nature of these conditions has been indicated.

SUMMARY

This article is not an evaluation of fluorescence microscopy, but merely a discussion of the basic factors of the technique.

1. The excitation band of auramine-O in dry gelatin has been determined to lie between 3400 Å and 4700 Å, with a broad maximum in the region of 4500 A. The excitation band of thioflavin is essentially similar, but the fluorescence a trifle less intense, so that thioflavin offers no advantages as the fluorescent dye for microscopy.

2. The wave-band of fluorescence of auramine in dry gelatin extends approximately from 5400 Å to 6500 Å.

3. The fluorescent efficiency or auramine in dry gelatin is extremely low, at best not over 0.02 per cent.

4. Glass of proper transmission characteristics and low thermal expansion is not available for light filters. Cuproammonium solution in a jar has been found the most practical substitute.

5. Glass for use in yellow ocular filters is available from several sources.

6. No satisfactory light source is available for fluorescence microscopy of the tubercle bacillus. Types of light source are discussed and an improvised illuminator is described.

7. Condensing lenses, filter jar and microscope condenser must transmit the excitation wave-band freely for good results. High power objectives that fluoresce strongly cannot be used for identification of *M. tuberculosis*. Most 4 mm. objectives are useless. An 8 mm. objective with 10x ocular is recommended for search and for identification of the organism.

8. The staining technique is described. Fresh stain is important.

9. The advantages and disadvantages of the method are listed.

SUMARIO

Este trabajo no constituye una justipreciación de la fluoromicroscopia sino meramente una discusión de los factores fundamentales de la técnica.

1. La franja de excitación de la auramina-O en gelatina seca, según se ha determinado, queda entre 3400 Å y 4700 Å, alcanzando un máximo de amplitud en la región de 4500 Å. La franja de excitación de la tioflavina viene a ser semejante, pero la fluorescencia es algo menos intensa, de modo que dicha sustancia no presenta ninguna ventaja como colorante fluorescente para empleo en microscopia.

2. La franja de fluorescencia de la auramina en gelatina seca se extiende aproximadamente de 5400 Å a 6500 Å.

3. La eficacia fluorescente de la auramina en gelatina seca es sumamente baja, cuando más sin pasar de 0.02 por ciento.

4. No se encuentra disponible cristal para filtros de luz que posea características adecuadas de transmisión y de poca expansión térmica. La solución de cuproamonio en un tarro ha resultado ser el sustituto más práctico.

5. Puede obtenerse de diversas partes cristal para empleo en los filtros oculares amarillos.

6. No se ha encontrado ningún foco luminoso satisfactorio para la fluoro-

microscopia del bacilo tuberculoso. Discútense aquí los tipos de focos luminosos y se describe un iluminador improvisado.

7. Las lentes condensadoras, el tarro-filtro y el condensador del microscopio deben transmitir la franja de excitación sin obstáculos para obtener buenos resultados. Para la identificación del *M. tuberculosis* no pueden utilizarse objetivos de mucho aumento que fluorescen intensamente, y la mayor parte de los de 4 mm. resultan inútiles. Para la pesquisa e identificación del microbio recomiéndase un objetivo de 8 mm. con un ocular de 10.

8. Descríbese la técnica de coloración. Es importante que los colorantes sean recientes.

9. Enuméranse las ventajas e inconvenientes de esta técnica.

REFERENCE

(1) GRAHAM, C. F.: Simplification of fluorescence microscopy, J. Lab. & Clin. Med., 1942, *27*, 1188.

DEMONSTRATION OF TUBERCLE BACILLI BY FLUORESCENCE MICROSCOPY[1]

A Comparative Evaluation of the Fluorescence and Ziehl-Neelsen Methods in the Routine Examination of Smears for Acid-fast Bacilli

DAVID G. FREIMAN AND GERTRUDE F. MOKOTOFF

Recent reports on the detection of tubercle bacilli in smears by means of fluorescence (1, 2, 3, 4, 5, 6) have stressed the greater number of positive smears found and the amount of time to be saved by using this new method. The present study was undertaken to determine the sensitivity of this method by comparing its performance with that of the Cooper modification of the Ziehl-Neelsen method, to determine whether any additional positive specimens revealed by fluorescence could be confirmed by other means, particularly by culture methods, and to estimate the amount of time, if any, that may be saved by using the fluorescence technique.

To this end, a series of 864 specimens of sputum and gastric contents was studied. With the exception of about 25 specimens, all were from patients with pulmonary tuberculosis whose sputum or gastric contents had been found positive for acid-fast organisms on smear or culture at some time or other. The remaining specimens were obtained from 10 patients who had radiographic evidence of tuberculosis but persistently negative sputum, 3 with pleural effusion also with negative sputum, 3 with chronic bronchiectasis and one with carcinoma.

Duplicate smears were made of each specimen, by contact whenever possible. One of these was stained by the Ziehl-Neelsen method using the Cooper modification (7) with brilliant green 1:5000 as a counterstain; the other was stained with auramine-O using the staining method described by Richards and Miller (1) and examined by fluorescence using the apparatus described by these authors. Every slide was examined for a period of five minutes, the amount of time routinely allotted to each slide in this laboratory. It has been found that the small increase in the number of positive smears obtained by more prolonged examination is not commensurate with the extra time spent, especially in view of the frequent sputum examinations performed on all patients and the routine use of culture methods.

The results of these examinations are summarized in table 1. Of the 192 direct sputum smears studied, 65.6 per cent were positive by the Ziehl-Neelsen method and 67.7 per cent by fluorescence, a very slight advantage of 3 per cent for the latter method. Only 4 sputa were positive by the Ziehl-Neelsen method alone, whereas there were 8 positive by fluorescence alone. On the other hand, in the study of 461 sputum concentrates, 21.5 per cent were positive by the Ziehl-Neelsen method and 20.6 per cent by fluorescence, thus giving an equally slight advantage of 4 per cent to the former over the latter method. In this group there were 24 smears positive by fluorescence alone as compared with 28 positive by

[1] From the Montefiore Hospital Country Sanatorium, Bedford Hills, New York.

Ziehl-Neelsen alone. In neither of the groups mentioned can the differences be considered significant. In the group of 211 gastric concentrates, however, appreciable differences were found, there being nearly twice as many positive smears found by fluorescence as by the Ziehl-Neelsen method. Whereas 7.1 per cent of the gastric smears examined were positive by the latter method, 13.7 per cent were found positive by the former, an increase of 93 per cent and, although 16

TABLE 1

Comparison of the number of smears found positive by the fluorescence and Ziehl-Neelsen methods

	TOTAL SPECIMENS EXAMINED	SPECIMENS POSITIVE BY BOTH METHODS COMBINED		SPECIMENS POSITIVE BY ZIEHL-NEELSEN			SPECIMENS POSITIVE BY FLUORESCENCE			ADVANTAGE OF FLUORES. OVER Z-N METHOD
		Number	Per cent	Number	Per cent of total specimens examined	Per cent of total positive specimens	Number	Per cent of total specimens examined	Per cent of total positive specimens	
										per cent
Sputum direct smear	192	134	69.8	126	65.6	94.0	130	67.7	97.0	+3
Sputum concentrate	461	123	26.7	99	21.5	80.5	95	20.6	77.2	−4
Gastric concentrate	211	31	14.2	15	7.1	48.4	29	13.7	93.6	+93
Total	864	288	33.3	240	27.8	83.3	254	30.0	88.0	

TABLE 2

Results of examination of duplicate sputum smears stained by the Ziehl-Neelsen method

	TOTAL NUMBER OF SPECIMENS EXAMINED	TOTAL NUMBER OF SPECIMENS FOUND POSITIVE ON AT LEAST ONE SMEAR	SPECIMENS FOUND POSITIVE ON ONLY ONE SMEAR AFTER FIVE MINUTES		SPECIMENS FOUND POSITIVE ON ONLY ONE SMEAR AFTER TEN MINUTES	
			Number	Per cent of total positive specimens	Number	Per cent of total positive specimens
Direct smears	100	58	0	0	0	0
Sputum concentrates	411	104	13	12.5	6	5.8

slides were positive by fluorescence alone, only 2 were positive by Ziehl-Neelsen alone.

Because of the likelihood that a portion of the differences in number of positive smears found by the two methods might be attributable to the fact that two smears were being examined in place of the usual one, a series of duplicate sputum smears was examined, using the Ziehl-Neelsen staining method for both (table 2). In a group of 100 pairs of direct sputum smears, prepared by contact and stained at the same time, there was complete agreement in the number of positive smears found. There was, however, considerable variation in the number of organisms

seen in a few of the pairs. On the other hand, in a group of 411 duplicate smears of sputum concentrates, also prepared by contact and stained simultaneously, 104 were found positive on at least one of the slides in the five-minute period of examination, but, of these, 13, or 12.5 per cent, were positive on one slide only. Since half of these can be expected to be positive on the first smear examined, it would appear that, in the case of sputum concentrates at any rate, a difference of at least 6 per cent in the number of positives found in two series of slides prepared from the same sputa cannot be considered significant for a five-minute examination. This is one and one-half times the percentage difference obtained by comparison of the sputum concentrates by the two methods. When the slides were examined for ten minutes, the percentage difference diminished by slightly more than half.

Attempts were made to confirm all specimens found positive by fluorescence but negative by the Ziehl-Neelsen method. In all such instances the position of the acid-fast organism or organisms found by fluorescence was recorded by means

TABLE 3

Confirmation of specimens positive by fluorescence but negative by the Ziehl-Neelsen method

	NUMBER CULTURED AND/OR RESTAINED	SPECIMENS CONFIRMED	
		Number	Per cent
Sputum direct smears	7	7	100
Sputum concentrate	21	14	67
Gastric concentrate	14	8	57
Sputum and gastric concentrates combined	35	22	63
Concentrated specimens, cultured	32	18	56

of vernier readings on the mechanical stage, and the slide was then restained by the Ziehl-Neelsen method and reëxamined. All those specimens which were not confirmed by restaining, together with some of those which were, were cultured on a minimum of 4 tubes of the modification of Petragagni's medium described in Diagnostic Standards (8). The results obtained in these attempts at confirmation are shown in table 3; a few specimens in which the culture medium became totally contaminated have been excluded from the series.

A total of 42 specimens positive by fluorescence only was studied in this way. Of these, 7 were positive on direct smear and all were confirmed. In only one of these instances was culture of the specimen necessary, however; the remainder were confirmed by restaining or by further examination of the smear stained by the Ziehl-Neelsen method. Of the remaining 35 specimens, consisting of sputum and gastric concentrates, 22, or 63 per cent, were confirmed by culture or restaining. This percentage is less than that obtained by culturing a group of similar specimens positive by the Ziehl-Neelsen method on the same medium and using the same technique, 211 out of 252 specimens, or 83.7 per cent, being positive on culture (table 4); it is almost the same as the percentage of posi-

tive cultures obtained from a group of 58 sputum and gastric concentrates in which only one to three organisms were found after a five-minute search (40 out of 58, or 69 per cent positive, table 4). Even if those specimens which were confirmed by restaining are excluded, and only those confirmed by culture methods compared (18, or 56 per cent, of 32 cultured sputum and gastric concentrates positive by fluorescence only were positive on culture[2] (table 3)), the difference between this figure and that obtained by culturing specimens weakly positive by Ziehl-Neelsen (69 per cent) is not sufficiently significant in view of the small numbers of specimens involved to permit any conclusions to be drawn.[3] It must be noted, however, that in two instances definite acid-fast rods, unconfirmed by culture, were found by fluorescence in specimens from patients whose sputum had never before been found positive either by smear or culture methods. One was obtained from a young girl with a very small minimal lesion, presumably tuberculous, in the left lower lobe. Numerous examinations of sputum and

TABLE 4

Results obtained by culturing specimens found positive by the Ziehl-Neelsen method after concentration

	NUMBER OF SPECIMENS CULTURED	POSITIVE CULTURES	
		Number	Per cent
Moderately or weakly positive specimens..	252*	211	83.7
Specimens in which only 1–3 organisms were found in five-minute search.....	58	40	69

* This figure includes the 58 specimens listed below.

gastric contents performed before and afterwards were consistently negative for acid-fast organisms. The other specimen was obtained from a woman with chronic bronchiectasis in whom no evidence of active tuberculosis was ever found. On the other hand, Bogen (3) cultured 250 specimens negative by the Ziehl-Neelsen method including 29 that were positive by fluorescence, and of this latter group 28 were found to be positive on culture. Van Dyke (6) reports similar results; of 41 specimens positive by fluorescence only, 40 were confirmed by culture.

To determine further the sensitivity of the fluorescence method, all specimens in which there was the slightest doubt as to whether a rod seen was or was not an acid-fast organism were also cultured (table 5). It was felt that, using strict criteria with a highly sensitive method, a number of specimens which were actually positive would be considered doubtful. Only 38 per cent of 50 such specimens were positive on culture, however, a figure practically the same as that obtained by culturing a group of 116 specimens from known tuberculous patients

[2] Two specimens positive after restaining were negative on culture.

[3] The observed difference in per cent is 1.21 times the standard deviation of differences in per cent to be expected if the two series were identical. Chance fluctuations alone can be expected to result in such a difference in 23 per cent of all cases.

who were found negative by both the fluorescence and Ziehl-Neelsen methods (36.2 per cent, table 6).

The results obtained in comparing the length of time required to find an acid-fast organism in smears found positive by the two methods used are shown in table 7. Except for minor differences the time required was practically the same,

TABLE 5

Confirmation of specimens negative by Ziehl-Neelsen but doubtful by fluorescence

	NUMBER CULTURED	SPECIMENS CONFIRMED	
		Number	Per cent
Sputum direct smears	4	2	50
Sputum concentrate	28	11	40
Gastric concentrate	18	6	33
Sputum and gastric concentrates combined	46	17	37

TABLE 6

Results obtained by culturing specimens found negative by both the fluorescence and Ziehl-Neelsen methods after concentration

	NUMBER OF SPECIMENS CULTURED	POSITIVE CULTURES	
		Number	Per cent
Sputum concentrate	51	17	33.3
Gastric concentrate	115	44	38.3
Total	166	61	36.7

TABLE 7

Comparison of time required to find an acid-fast organism on positive smears by the fluorescence and Ziehl-Neelsen methods

METHOD	TOTAL NUMBER OF POSITIVE SMEARS TIMED	FIRST ACID-FAST ORGANISM FOUND IN									
		1st minute		2nd minute		3rd minute		4th minute		5th minute	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Ziehl-Neelsen	234	185	79	20	8.6	10	4.2	9	4.0	10	4.2
Fluorescence	253	192	76	20	8.0	19	7.5	12	4.5	10	4.0

more than three-fourths of the positive smears being discovered within the first minute of examination. No time seems to be saved, therefore, by using the fluorescence technique, at least for the examination period involved. The time-saving advantages claimed for the method are based on the fact that a larger field can be covered in a given time; but this is, at least in part, counterbalanced by the disadvantages of the lower power of the objective used (4 mm. instead of

oil-immersion), the difficulties attendant upon focusing and checking the focus and the tendency to examine too rapidly a dim field in which little or nothing is to be seen.

From the technical point of view the method has certain disadvantages. The greatest difficulty encountered was in focusing on the slide and in remaining in focus throughout the examination when the surface of the slide examined was uneven. A drop of auramine was placed at the edge of each slide to facilitate initial focusing. It was also found that a slight residue of dye particles on the smear or a faintly luminescent background was advantageous since the focus could then be checked at frequent intervals. Too much dye on the slide was an interfering factor, however; it slowed the examination considerably and undoubtedly diminished its accuracy. Sometimes this was unavoidable, since with certain sputa, no amount of decolorizing seemed to be sufficient. In general, there were few interfering factors, provided that the stain was fresh and the slides clean.

TABLE 8

Results of routine culture of microscopically negative sputum and gastric specimens over a four-year period

YEAR	TOTAL SPECIMENS CULTURED	POSITIVE CULTURES	
		Number	Per cent
1939	697	266	38.2
1940	992	323	32.6
1941	1,094	338	30.8
1942	1,473	412	28.2
Total.....	4,256	1,339	31.5

It was also found that a fairly well darkened room was necessary for best results since accommodation was much better under these circumstances. Eye fatigue seemed to be somewhat greater than with the Ziehl-Neelsen method. Contrary to the impression gathered from previous reports, too, the method is not one to be mastered over night, but requires a fair amount of practice before reliable results are obtained.

On the credit side, there is the simplified staining procedure, requiring no steaming of the dye and no counterstain. In addition, more of the slide can be covered in a given time, although perhaps less thoroughly; isolated bits of sediment in thin smears are picked up more quickly than they are by the other method and there is likelihood that a greater number of such areas will be examined. Since many smears made from gastric concentrates are thin, it is perhaps for this reason that a considerable increase in positive smears was found by fluorescence in this group rather than in the others.

Whatever may be the advantages of the new method over the old, it is obvious that culture of the specimens is considerably more sensitive than either. Cul-

ture of those specimens found negative by both methods, practically all from tuberculous patients, indicated that at least 37 per cent of them were actually positive but were missed by microscopical methods, this increment by culture being, of course, indicative not only of the sensitivity of the culture method but of the quality of smear and concentrate as well. Analysis of the results obtained by culturing microscopically negative sputum and gastric specimens over a four-year period (table 8) reveals that of 4,256 such specimens, 1,339, or 31.5 per cent, were found to be positive, with monthly variations from as low as 20 to as high as 50 per cent.

Since the criteria for calling a rod seen by fluorescence a tubercle bacillus must be very strict, and since, in spite of the inconclusive results of culture, there remains a possibility that an occasional specimen will not be confirmed, the fluorescence method as now constituted seems to offer little advantage over the less exacting and more convenient Ziehl-Neelsen method. This is particularly true in this laboratory where persistently negative sputa and all gastrics are routinely cultured and where examinations are performed at frequent intervals.

SUMMARY AND CONCLUSIONS

The sensitivity of the fluorescence method in the examination of sputum and gastric smears for acid-fast bacilli was investigated by comparing its performance with the Cooper modification of the Ziehl-Neelsen method. A significant increase (93 per cent) in the number of positive specimens found by fluorescence was obtained only in the group of gastric concentrates. Differences observed in the examination of untreated and concentrated sputum specimens were too small to be significant and in the latter case were less than the differences obtained when a series of duplicate smears were examined by the Ziehl-Neelsen method alone. No conclusive difference could be demonstrated between the percentage of confirmation by culture or restaining of smears of specimens positive by fluorescence only and of specimens weakly positive by Ziehl-Neelsen, but the percentage of positive cultures obtained from a group of specimens considered doubtfully positive after examination by fluorescence was no greater than that obtained from a group found negative by both methods and the time required to find an acid-fast organism in a positive smear by either method was about the same. The fluorescence method appears to offer little advantage over the less exacting Ziehl-Neelsen method, especially in view of the fact that culture methods used routinely in this laboratory are considerably more sensitive than either.

SUMARIO Y CONCLUSIONES

Comparando los resultados con los obtenidos con la modificación de Cooper de la técnica de Ziehl-Neelsen se investigó la sensibilidad de la técnica de la fluorescencia en el examen del esputo y de los frotes gástricos en busca de bacilos ácido-resistentes. Sólo en el grupo de los concentrados gástricos observóse un aumento significativo (93 por ciento) en el número de ejemplares positivos descubiertos con la técnica fluorescente. Las diferencias observadas en el

examen de los ejemplares de esputo sin tratar y concentrados fueron demasiado pequeñas para revestir importancia, y en lo to cante a los últimos fueron menores que las obtenidas al examinar una serie de frotes duplicados con la técnica de Ziehl-Neelsen sola. No pudo distinguirse ninguna diferencia terminante entre el porcentaje confirmado por medio de cultivos o de la recoloración de los frotes de ejemplares que sólo fueron positivos con la técnica de la fluorescencia y los débilmente positivos a la Ziehl-Neelsen, pero el porcentaje positivo obtenido en un grupo de ejemplares considerados dudosamente positivos tras el examen con la técnica de la fluorescencia, no fué mayor que el obtenido en un grupo que resultó negativo con ambas técnicas; mientras que el tiempo necesario para descubrir un microbio ácido-resistente en un frote positivo con uno u otro método fué aproximadamente idéntico. La técnica de la fluorescencia parece poseer muy poca superioridad sobre la menos minuciosa de Ziehl-Neelsen, sobre todo visto que las técnicas de cultivo empleadas sistemáticamente en el laboratorio de los autores son mucho más sensibles que una u otra.

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AUTOLYSIS OF TUBERCLE BACILLI AND THE PRODUCTION OF TUBERCULIN (TUBERCULOPROTEIN)[1]

H. J. CORPER AND MAURICE L. COHN

Ever since its introduction in the nineties, tuberculin has occupied one of the most important places in the literature and in the practical and scientific phases of the chemistry of tuberculosis. Yet, biologically, the definition of its origin has not been clear: whether it is a metabolism or disintegration product of the bacilli or whether it is present as such in the intact bacilli.

As far back as 1918 (1), it was shown that tubercle bacilli of both human and bovine varieties possess autolytic enzymes, indicated by the noncoagulable nitrogen and amino-acid α nitrogen liberated at incubator temperature after the bacilli had been killed by toluol and chloroform. At that time, the trichloracetic acid coagulable nitrogen was not associated with tuberculin because the purpose of that study was primarily academic. Its aim was to discern the enzymes in the tubercle bacillus in the hope of using this information for preparing an antigen for complement fixation. Following this, it was pointed out that tubercle bacilli can survive for a long time when grown on relatively poor nutrient media (2); during this period, they show certain morphological changes in structure (cytomorphosis) (3), which parallel the curves of active growth, then pass into relative quiescence (or senescence) when certain specific materials are formed and liberated. With the development of simple nonprotein synthetic media, the tuberculoprotein was prepared in pure form and its characteristics recognized more fully. Little information was obtained on the mechanism of its production, however, beyond the tedious natural liberation occurring in growing cultures (4). In 1940 (5), it was noted that the tuberculoprotein present in the liquid medium is not appreciable in 500 cc. volume of Wong-Weinzirl medium (6) in 1 liter bottles until after maximum growth of human tubercle bacilli has been approached at about two to three months after planting. The liberation of tuberculoprotein into the liquid medium increases gradually to a maximum with the aging of the bacillary mass in from six to ten months and probably is a product of autolysis of the bacilli. The same year, Wong and Ouyang (7) succeeded in increasing the yield of tuberculin (tuberculoprotein) in liquid cultures by adjusting the pH to the optimum alkaline side without increase in culture production. They believed this favored rapid cell autolysis and inferred this tuberculin consisted essentially of bacillary (or cellular) proteins besides metabolic tuberculin. The tuberculin was prepared by suspending the decanted bacilli in 0.05 normal sodium hydroxide and incubated until the majority of bacilli were not stainable by the Ziehl-Neelsen technique. Autolysis was noted to appear on the third day of incubation in the alkaline solution and was practically completed on the fourteenth day, while no disintegration was noted in distilled water. The preparation was neutralized with N hydrochloric acid to a final pH 6.0 to 6.5 and was heated at 100°C. for three hours before trichlor-

[1] From the Research Department, National Jewish Hospital, Denver, Colorado.

acetic acid precipitation. The total nitrogen of the completed preparation (trichloracetic acid precipitate) was 13.27 per cent, which is the same as when prepared directly from the culture medium. Whether this preparation was the result of autolysis or hydrolysis in alkaline solution was not proved by this study.

PRELIMINARY EXPERIMENTS

Effect of Hydrogen-ion Concentration and Temperature on Liberation of Tuberculoprotein in the Presence of Toluene and Potassium Cyanide

In one of the early experiments of this study, one month old cultures of avirulent human tubercle bacilli grown on liquid Wong-Weinzirl medium were filtered through a sterile filter paper on a Buchner funnel and washed with sterile distilled water until wash water was free from chlorides when tested with silver nitrate. The one month old culture was used because it contains very little tuberculoprotein in solution at this age. The washed bacillary mass was then transferred to sterile bottles containing 100 cc. sterile buffer solutions of the following pH: 5.0, 6.0, 7.0, 8.0 and 9.0. The pH 5.0 to 8.0 buffer solutions were prepared according to the phosphate mixtures of Sørensen, described in *The Determination of Hydrogen Ions* by Clark, while the pH 9.0 buffer was the Palitsch's borax-boric acid mixture. These mixtures of bacilli and buffers were then incubated at 37°C. using one set, the "control," as such without further additions; another set, the "autoclaved," had been autoclaved for thirty minutes at 20 lbs. pressure before incubation; a third set, the "toluol" treated, to which 1 cc. of C. P. toluol had been added and vigorously shaken at frequent intervals during incubation; and a fourth set, the potassium cyanide "KCN" treated, to which 0.1 g. of C. P. potassium cyanide had been added. The "control" and "autoclaved" were used as controls, the former to discern the natural liberation from viable bacilli and the latter to discern any liberation from heat killed bacillary bodies due to hydrolytic action; while the "toluol" treated test was to discern the effect of autolysis, and the "cyanide" treated to discern the effect of enzyme intoxication or paralysis. The latter, however, did not succeed because the cyanide did not kill the bacilli in the concentration used, as will be noted from the following results. Samples taken at various intervals of incubation from the toluol and cyanide treated sets, as well as the controls, in the various buffers disclosed that the bacilli remained viable in all the buffer solutions for one month except the Palitsch's borax-boric acid buffer at pH 9.0 in which case the bacilli remained alive only up to the fifth day of incubation. In the case of the toluol, the bacillary mass was evaporated to dryness in a vacuum desiccator over phosphoric anhydride to remove the toluol (which treatment is innocuous to the bacilli since the control removed immediately after mixing yielded positive egg-yolk cultures), and then the bacilli were suspended in saline for culturing. The toluol in the buffer was found to kill the bacilli within one day in all cases. With the cyanide treated buffer, the mixture was acidified with 3 per cent hydrochloric acid (to liberate hydrocyanic acid) before vacuum evaporation. It was found that the cyanide was unable to kill the tubercle

bacilli, since growth was obtained on egg-yolk medium up to one month of contact at incubator temperature.

In order to determine the amount of trichloracetic acid precipitable material liberated from the bacilli under the various conditions of experiment, a sample was withdrawn under sterile precautions from each bottle. Then from 1 to 5 cc. of the centrifuged clear supernatant liquid was placed in a Hopkins graduated tube and an equal volume of 20 per cent C. P. trichloracetic acid solution was added. The resultant precipitate was measured and, for tabulation purposes, was transposed to milligrams of tuberculoprotein using the Seibert factor, 0.0674. The pertinent results of these analyses are recorded in table 1, which includes the pertinent findings at pH 5.0, 7.0, and 8.0.

TABLE 1

The liberation of tuberculin from washed tubercle bacilli

pH	REAGENT AND TREATMENT	TIME OF INCUBATION AND MILLIGRAMS OF TUBERCULIN PER CUBIC CENTIMETER IN CENTRIFUGED LIQUID					
		1 day	2 days	4 days	1 week	2 weeks	4 weeks*
5	Control	0.08	0.11	0.15	0.16	0.16	0.16
	Autoclaved	0.19	0.19	0.20	0.18	0.19	0.19
	Toluol	0.11	0.32	0.51	0.66	0.66	0.67
	KCN	0.08	0.11	0.16	0.18	0.28	0.30
7	Control	0.16	0.23	0.26	0.29	0.28	0.38
	Autoclaved	0.38	0.38	0.37	0.37	0.38	0.38
	Toluol	0.23	0.55	1.05	1.61	1.48	1.50
	KCN	0.12	0.16	0.19	0.19	0.51	0.53
8	Control	0.16	0.23	0.24	0.43	0.52	0.54
	Autoclaved	0.40	0.40	0.42	0.43	0.48	0.49
	Toluol	0.19	0.50	1.08	1.50	1.61	1.61
	KCN	0.13	0.16	0.35	0.46	0.65	0.66

* The findings in the toluol treated tests remained practically stationary after four weeks.

It is to be noted from the findings recorded in table 1 that during the four weeks' observation period there is very little increase in the tuberculin obtained from the bacilli (control) at pH 5.0 at 37°C.; while at pH 7.0 and 8.0, this is increased perceptibly. In the autoclaved bacilli, though showing a slightly greater amount of tuberculin initially, the amount of tuberculin remained practically constant throughout the four weeks at pH 5.0, 7.0, and 8.0. The most striking effect is noted for the bacilli treated (killed) with toluol, in which case at pH 7.0 and 8.0 a markedly accelerated liberation of tuberculin occurs for the first week. At pH 5.0, this acceleration is not so evident. All the evidence in this experiment points to the fact that the liberation of the tuberculin from the bacilli is caused by autolysis and the presence of tuberculo-bacillary autolytic enzymes account for this action. These enzymes are destroyed by heat (auto-

claving), since autoclaved bacilli treated with toluol show no accelerated liberation of tuberculin at incubator temperature. It is obvious from the cyanide and the control tests that the bacilli must be killed before the autolytic enzymes can act at maximum efficiency.

A study of the effect of temperature on the liberation of tuberculin by autolysis further emphasizes the autolytic enzymatic action in its production. The results of such an experiment with toluol and bacilli at various temperatures are recorded in table 2.

The data recorded in table 2 give the results in milligrams of tuberculoprotein equivalent and were calculated from the volume of trichloracetic acid precipitate by using the Seibert factor of 0.0674. The table shows that the autolysis of tubercle bacilli with liberation of tuberculin proceeds best at incubator temperature (37°C.); and at refrigerator temperature (3°C.), practically no autolytic disintegration results; while at room temperature (24° to 30°C.), it was definitely retarded.

TABLE 2

The effect of temperature on the autolytic liberation of tuberculin

TEMPERATURE*	TIME INCUBATED AND MILLIGRAMS OF TUBERCULOPROTEIN PER CUBIC CENTIMETER IN CENTRIFUGATED LIQUID				
	1 day	2 days	4 days	1 week	2 weeks
Incubator 37°C.	0.23	0.39	0.70	1.48	1.55
Room 24–30°C.	0.07	0.09	0.12	0.27	0.55
Refrigerator 3°C.	0.04	0.04	0.04	0.05	0.05

* All human tubercle bacilli (one month old washed cultures from Wong-Weinzirl Medium) were suspended in 100 cc. Sørensen's phosphate mixture buffer, pH 7.0, and 1 cc. C. P. toluol.

In order to ascertain the amount of tuberculin liberated by autolysis from a definite amount of tubercle bacilli, a single large one-month old culture of human tubercle bacilli, grown on 500 cc. of Wong-Weinzirl medium, was filtered on a Buchner funnel through a filter paper and was thoroughly washed (free from chlorides in the wash water) and then dried in a vacuum desiccator over phosphorus pentoxide to constant weight which was 2.379 g. These dried bacilli were suspended in 100 cc. of Sørensen's phosphate buffer, pH 7.0, with 1 cc. C. P. toluol at incubator (37°C.) temperature for one week; the trichloracetic acid precipitate was determined with a Seibert figure of 155 mg. of tuberculin as the yield. This was 6.5 per cent of the original dried bacillary mass, which possesses a high biological activity in tuberculo-allergic sensitized animals, both for skin test and intoxication, as will be shown later.

NATURE AND COMPOSITION OF THE TUBERCULIN LIBERATED BY AUTOLYSIS

Since ultrafiltration gives a simple approximation of particle size (8), this method was used to determine the particle size of the tuberculin obtained from tubercle bacilli by autolysis. All the factors entering into ultrafiltration, how-

ever, must be recognized in making interpretations from these results. However, ultrafiltration was used in this study, primarily to remove bacillary bodies without too greatly disturbing the factor of quantitative tuberculin filtration, such as occurs as a result of adsorption when Seitz filtration is used. A study of various concentrations of collodion membranes used for testing revealed that viable tubercle bacilli could be removed completely and readily from suspensions when the filtration membrane had been prepared from 1.5 per cent collodion, using acetic acid as solvent. The effects of filtration through various percentages, from 1.5 to 5.0 per cent collodion membranes (in Whatman No. 1 filter paper), are recorded in table 3. The tuberculin filtered was prepared in each case from a separate one-month culture grown on 500 cc. Wong-Weinzirl medium. The bacillary mass was filtered and washed, and finally suspended

TABLE 3

The effect of ultrafiltration on the recovery of tuberculin prepared by autolysis

PER CENT COLLODION	DIAMETER OF RETAINED PARTICLES IN $\mu\mu$*	MILLIGRAMS OF TUBERCULOPROTEIN PER CC. IN CENTRIFUGATED LIQUID	MILLIGRAMS OF TUBERCULOPROTEIN PER CC. IN FILTRATE	PER CENT LOSS BY FILTRATION	TUBERCULOPROTEIN IN FILTRATE (50 CC.) CALCULATED	TUBERCULOPROTEIN IN FILTRATE (50 CC.) RECOVERED	PER CENT NITROGEN IN TUBERCULOPROTEIN
1.5	480	1.75**	1.28**	27	64	61	15.50
2.0	350–370	1.61	1.21	25	61	64	15.02
2.5	242–250	1.41	0.94	33	47	44	15.10
3.0	150–168	1.41	0.67	52	33	29	15.18
3.5	90–100	1.41	0.40	71	20	21	14.87
4.0	60–68	1.41	0.27	81	13	11	14.93
4.5	35–38	1.61	0.23	86	11	9	14.88
5.0	19–22	1.55	0.19	88	9	5	14.82

* The diameter of retained particles by ultrafiltration is taken from the article by Kreuger and Ritter (8).

** These figures are obtained from the computation of the amount of tuberculoprotein, using the Seibert factor, and the volume of trichloracetic acid precipitate.

in 100 cc. Sørensen's phosphate buffer, pH 7.0, to which was added 1 cc. C. P. toluol and the mixture incubated at 37°C. in a stoppered bottle for one week.

The findings recorded in table 3 indicate that a gradual diminution in the protein passing through the ultrafilter occurs with gradual decrease in pore size. This suggests that tuberculin as derived from autolysis of tubercle bacilli is not of uniform particle size but rather consists of a mixture of tuberculins varying in particle size from 480 $\mu\mu$ to 19 to 20 $\mu\mu$ which practically removes about 88 per cent of the tuberculin. It is to be noted also that the Seibert factor, 0.0674 times the volume, gives a calculated figure which is consistent with what actually is obtained by gravimetric methods. The percentage nitrogen, which ranges from 15.50 to 14.82, is higher than when tuberculin is prepared by natural decomposition of the bacilli in the culture flask, which ranges from 13.0 to 14.0 per cent nitrogen (also recorded by Wong and Ouyang).

BIOLOGICAL ACTIVITY OF TUBERCULIN PREPARED BY AUTOLYSIS

In determining biological activity of tuberculin, there are three different methods which can be used: (*1*) the skin reaction in a tuberculo-allergic animal either produced by virulent bacillary infection by the injection of relatively

TABLE 4

Amount of tuberculoprotein required for reaction

TEST USED*	NATURAL SEITZ FILTRATE (FIGURE IS TUBERCULOPROTEIN CONTENT)	TRICHLORACETIC ACID PRECIPITATE FROM NATURAL SEITZ FILTRATE	FILTRATE FROM AUTOLYSIS OF WASHED BACILLI (1.5 PER CENT COLLODION ULTRAFILTRATION)	TRICHLORACETIC ACID PRECIPITATE (FROM AUTOLYSTATE OF WASHED BACILLI)
Tuberculin (intracutaneously)	0.000,05 mg. (guinea pig sensitized with heat-killed bacilli in oil)	0.000,05 mg. tuberculoprotein	0.000,05 mg. tuberculoprotein	0.000,05 mg. tuberculoprotein
Anaphylactic sensitization	1 mg. tuberculoprotein or in repeated injections of 0.0009 mg. tuberculoprotein		1 mg. tuberculoprotein or in repeated injections of 0.0009 mg. tuberculoprotein	
Anaphylactic intoxication	0.3 to 1.3 mg. of tuberculoprotein, depending on amount of sensitization	1 to 2 mg. tuberculoprotein**	0.3 to 1.3 mg. of tuberculoprotein, depending on amount of sensitization	1 to 2 mg. tuberculoprotein
General allergic intoxication	Lethal, depending on amount of tuberculous or allergic sensitization (with viable avirulent or heat-killed bacilli in oil)	About identical to column 2 in amount (tuberculoprotein) required for lethal effect	About identical to column 2 in amount (tuberculoprotein) required for lethal effect	About identical to column 2 in amount (tuberculoprotein) required for lethal effect

* All these tests were performed on guinea pigs which either had been sensitized over one month prior to second injection or where sensitization was tested one month or more elapsed before second injection and final test was made.

** See also Corper, H. J., and Cohn, Maurice L.: Intoxication in tuberculosis, Am. Rev. Tuberc., 1940, *41*, 71.

large amounts of viable avirulent tubercle bacilli or by heat killed bacilli in relatively large amounts suspended in mineral oil; (*2*) the general allergic intoxication reaction following the injection by any route—intravenous, subcutaneous or intraperitoneal, in guinea pigs made tuberculo-allergic as in (*1*) and determining the minimal lethal amount with due consideration to the

tuberculo-allergic condition of the animal (9); and (*3*) anaphylactic sensitization and intoxication.

These were applied (*1*) to the natural Seitz filtrate from the growth of tubercle bacilli on the Wong-Weinzirl liquid medium for over three months, (*2*) to the trichloracetic acid precipitate prepared from this natural Seitz filtrate, (*3*) to the filtrate obtained through a 1.5 per cent collodion membrane from washed tubercle bacilli autolyzed under toluol for one week at incubator temperature (37° C.) and (*4*) to the trichloracetic acid precipitate prepared from the foregoing filtrate from the autolyzed bacilli.

The summarized comparative results of these tests are recorded in table 4.

The data recorded in table 4 suggest strongly the identity of the biological substances (*1*) obtained from natural Seitz filtrate, resulting from the growth of tubercle bacilli on a nonprotein synthetic medium (Wong-Weinzirl) at incubator temperature for over three months; (*2*) those obtained from the autolysate of washed tubercle bacilli at incubator temperature for one week; and (*3 and 4*) the trichloracetic acid precipitated protein obtained from these solutions (*1*, the natural filtrate or *2*, the autolysate). This biological identity is emphasized by the production of anaphylactic sensitization and intoxication, the local tuberculin skin reaction and general allergic intoxication in tuberculous and tuberculo-allergic hypersensitive guinea pigs. The tuberculo-allergic hypersensitiveness in these guinea pigs is produced by the injection of viable avirulent human tubercle bacilli or heat-killed tubercle bacilli suspended in mineral oil.

A COMPARISON OF THE VALUE OF VARIOUS PURE CHEMICALS FOR AUTOLYZING TUBERCLE BACILLI

The various reagents or pure chemicals recommended in the past as suitable for autolysis or plasmolysis are amylalcohol, benzene, benzyl alcohol, bromobenzene, carbon disulfide, carbon tetrachloride, chloroform, diethyl ether, petroleum ether, toluene, trichloro-ethylene and xylene. Since in the case of tubercle bacilli, however, it appeared essential that these reagents be capable of destroying viability, the problem of autolysis resolved itself into two factors to be determined: one, the viability of the bacilli, or rather the ability of the reagent to destroy viability; and the second, the autolysis of the bacilli with a view to the liberation of tuberculoprotein (tuberculin) in suitable and available form. The results of an experiment with the foregoing reagents giving the yield of tuberculoprotein liberated at various time intervals is presented in table 5.

An examination of the data recorded in table 5 indicates that the best results, both in destroying the viability of the tubercle bacilli and in yielding tuberculoprotein from the autolysis of these bacilli, were attained from toluene during the course of one week. The results with benzene, bromobenzene, carbon disulfide, and trichloro-ethylene also proved satisfactory, both with regard to destroying the viability of the bacilli within one to two days and giving a good yield of tuberculoprotein. Diethyl ether and petroleum ether did not destroy the tubercle bacilli within a month, while chloroform and carbon tetrachloride proved unserviceable because of the tendency to precipitate the tuberculoprotein. Ben-

zyl alcohol proved unsatisfactory because it could not be removed readily and the yield of tuberculoprotein was small.

TABLE 5

The effect of various pure chemicals on the viability and autolysis of tubercle bacilli

REAGENT	TIME AND VOLUME* OF TRICHLORACETIC ACID PRECIPITATE FROM 5 CC. OF CENTRIFUGATED LIQUID					
	1 day	2 days	4 days	1 week	2 weeks	1 month
Amyl alcohol	0 V****	2.5	6.7	8.1	11.5	12.0***
Benzene	2.9	6.1	8.9	15.2	12.7	13.9
Benzyl alcohol	0.7	1.5	3.1	3.2	3.2	4.5
Bromo benzene	1.5	3.1	7.6	10.7	23.0	20.5
Carbon disulfide	1.3	4.8	7.9	8.2	13.8	12.3
Carbon tetrachloride	1.8	4.6	5.8	3.3	2.5	2.5
Chloroform	1.6	5.1	5.1	5.1	6.6	9.2
Diethyl ether	0**V	Trace** V	Trace** V	8.6 V	10.8 V	7.8 C!V
Petrol ether	3.1 V	9.0 V	11.3 V	11.3 V	9.5 V	10.9 V
Toluene	1.8 V	8.2	12.1	12.1	12.1	10.6
Trichloro-ethylene	2.0 V	3.6V	7.1	8.1	20.2	18.1
Xylene	1.3	2.4	4.8	5.5	12.6	14.3

* Calculated on the basis of 8.9 grams of moist culture in each reaction mixture. The figures given are readings on the Hopkin's tubes in divisions of 0.01 cc.

** Cloudy but no centrifugable precipitate.

*** All reactions of liquids were pH 7 at one month.

**** V indicates culture still viable at time shown.

!C = contaminated.

Note: In a glass sealed tube the bacilli are killed by diethyl ether and petroleum ether within three days at 37°C.

AN ATTEMPT TO DETERMINE THE NATURE OF THE AUTOLYTIC ENZYME ACTION IN TUBERCLE BACILLI

In previous studies, it was noted that heat-killed tubercle bacilli, unlike viable bacilli, would not autolyze in the presence of toluene, thus defining autolysis as an enzymatic process. Thus far it had not been determined whether the autolytic enzymes of tubercle bacilli were water soluble or could be obtained in the filtrate free of bacillary bodies. In an attempt to do this, the autolysate liquid from viable bacilli, destroyed with toluene and of known tuberculoprotein content, was mixed with heat-killed bacilli as substrate. The mixture was then incubated, and samples were withdrawn at various intervals up to one week to determine any change in tuberculoprotein content of the filtrate. The procedure used to prepare the enzyme filtrate was briefly as follows: (*a*) A one-month old culture (about 2 g. equivalent of vacuum dried bacillary mass) was removed from the medium (Wong-Weinzirl liquid) and the bacilli were washed until practically free from chlorides. To these were then added 100 cc. of distilled water, 50 mg. sodium carbonate and 1 cc. of toluene. The mixture was incubated at 37°C. for one week and was filtered through a 1.5 per cent gun cotton membrane. (*b*) The heat-killed bacilli were likewise taken from a one-month old culture (500 cc. Wong Weinzirl medium) and, after washing free from chlorides,

were transferred to a bottle containing 25 cc. of distilled water and 12.5 mg. sodium carbonate in which they were autoclaved for fifteen minutes at 20 lbs. pressure. After cooling, the filtrate from (*a*) was added, and in addition 1 cc. of toluene. The determinations for tuberculoprotein were made on centrifugated samples by using trichloracetic acid as precipitant added to 1 cc. in a Hopkins tube. The results in volume of precipitate were as follows: sample taken immediately was 1.6; after one day at incubator temperature, 1.6; after two days, 1.8; after five days, 1.6; and after one week, 1.7. This experiment was repeated in all details except that the heat-killed bacilli were replaced by an acetic acid killed culture (5 per cent for three days at 37° C.), and the acetic acid was removed by washing with sterile distilled water and decantation, after which the pH of the liquid was adjusted to about 7.5 by means of dilute sodium hydroxide. The readings resulting were as follows: immediately, 1.4; one day, 1.4; two days, 1.5; four days, 1.4; and at one week, 1.5.

It is noted from these experiments that no appreciably amount of enzymatic activity could be demonstrated in the liquid after autolysis of tubercle bacilli. However, it did not appear warranted to state that autolytic enzymes of the bacilli were not liberated, since it is conceivable that the products of autolysis themselves may have paralyzed these enzymes or that the heat or chemical (acetic acid) treatment of the bacilli may have altered the bacillary substrate to such an extent that these enzymes could not react on them. It seems likely, however, that the potent enzymes of the bacilli act mainly intracellularly on the bacilli and probably are not liberated in sufficient concentration in the filtrate to be able to act upon the proteins of the heat-killed or chemically-killed bacilli. The liberation of the tuberculoprotein into the solution *in vitro* may also account for the difference to some extent between *in vitro* liberation in cultures and its absence *in vivo* in man or animals.

SUMMARY

1. Tubercle bacilli grown on a simple nonprotein synthetic medium for one month and washed, autolyze (toluol) at incubator temperature (37°C.) at pH 7.0 to pH 8.0 to produce a maximum and highly potent biological tuberculin (tuberculoprotein) within one week.

2. The autolysis of human tubercle bacilli occurs best at incubator temperature (37°C.) and is practically completely retarded at refrigerator temperature (3°C.).

3. The autolysate obtained from human tubercle bacilli is biologically identical in activity, as determined by anaphylactic and allergic tests, to the tuberculin (tuberculoprotein) obtained in the natural filtrate from growing these same bacilli on a nonprotein synthetic medium for over three months.

4. This autolysate is free from contamination with extraneous constituents of the medium as well as foreign materials resulting from the growth of the bacilli.

SUMARIO

1. Los bacilos tuberculosos cultivados por un mes en un sencillo medio sintético no proteico, y lavados, se autolizan (tolueno) a la temperatura de la estufa

(37° C) a un pH de 8.0 para producir en una semana una tuberculina biológica máxima y potentísima (tubérculoproteína).

2. La autólisis de los bacilos tuberculosos humanos se obtiene mejor a la temperatura de la estufa (37° C) y se retarda casi por completo a la temperatura del refrigerador (3° C).

3. El autolisado obtenido de los bacilos tuberculosos humanos es biológicamente idéntico en su actividad, determinada por pruebas anafilácticas y alérgicas, a la tuberculina (tubérculoproteína) obtenida del filtrado natural procedente del cultivo de los mismos bacilos durante más de 3 meses en un medio sintético sin proteína.

4. Este autolisado está exento de contaminación por componentes extraños del medio, así como por materias extrañas procedentes del cultivo de los bacilos.

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TREATMENT OF EXPERIMENTAL TUBERCULOSIS IN THE VACCINATED AND NONVACCINATED GUINEA PIG WITH PROMIN[1]

W. STEENKEN, JR., F. H. HEISE AND E. WOLINSKY

The treatment of experimental tuberculosis with promin[2] (sodium salt of P,P′-diaminodiphenylsulfone-N,N′-didextrose sulfonate) has been reported by Feldman, Hinshaw and Moses (1, 2, 3, 4). They showed that promin retards the progression and favors better healing of the disease in the guinea pig, regardless of whether or not the drug is fed prior to, at the start of or after infection. Other investigators have had varying results. Woodruff (5) found that promin given subcutaneously was not beneficial to tuberculous guinea pigs. Steinbach and Duca (6) reported that this drug exerted a retarding effect on the course of experimental disease when given subcutaneously, but their results were not so clear-cut nor so dramatic as those of Feldman and his coworkers. Smith, Emmart and Westfall (7) showed fair results when promin was given intravenously to rabbits infected with bovine (Ravenal) tubercle bacilli, and also when fed to guinea pigs infected with a virulent human strain.

In the studies reported here we have repeated in part the work of Feldman, Hinshaw and Moses with slight variation. We have also studied the effect of the drug in guinea pigs that had been vaccinated with living attenuated tubercle bacilli H37 "R_a" variant.

METHODS

Sixty tuberculin-negative guinea pigs, of which 30 were males and 30 females, all weighing approximately 300 g. each, were divided into three groups as follows:

A: 20 untreated controls
B: 20 treated orally with promin
C: 20 vaccinated with living H37 "Ra" tubercle bacilli and treated orally with promin

Each animal in group C was vaccinated subcutaneously on three alternate days with 0.5 cc. of a suspension of tubercle bacilli containing 2.5 mg. of living H37 "R_a" microörganisms. Thus each animal received a total of 7.5 mg. Three weeks after the last vaccinating dose these animals were skin tested with Old Tuberculin and all reacted with marked areas of erythema and induration.

Groups B and C received orally, by syringe, 150 mg. of promin per day in two divided doses of 75 mg. each, one at 7 a.m. and the second at 5 p.m., starting two days prior to infection, and every day thereafter until the experiment was terminated in two hundred and twenty-four days.

Infection: Groups A, B and C were then infected subcutaneously in the left inguinal region with 0.5 cc. of a suspension of H37 "R_v" microörganisms that had

[1] From the Research and Clinical Laboratory, Trudeau Sanatorium, Trudeau, New York.

[2] Promin used in this study was kindly supplied through the courtesy of Dr. E. A. Sharp, Parke, Davis & Company, Detroit, Michigan.

been grown on Proskauer and Beck's synthetic medium for fourteen days and which contained 0.000,25 mg. weight, or about 10,000 organisms.

RESULTS

In the control group 12 animals died of tuberculosis. The first, on the fifty-ninth day, had a peritonitis in addition to generalized involvement of other organs, rating *12*.[3] One animal died (six days) of streptococcus pneumonia. One died (fifty-six days) of enteritis, rating *9*. Six were killed at the end of two

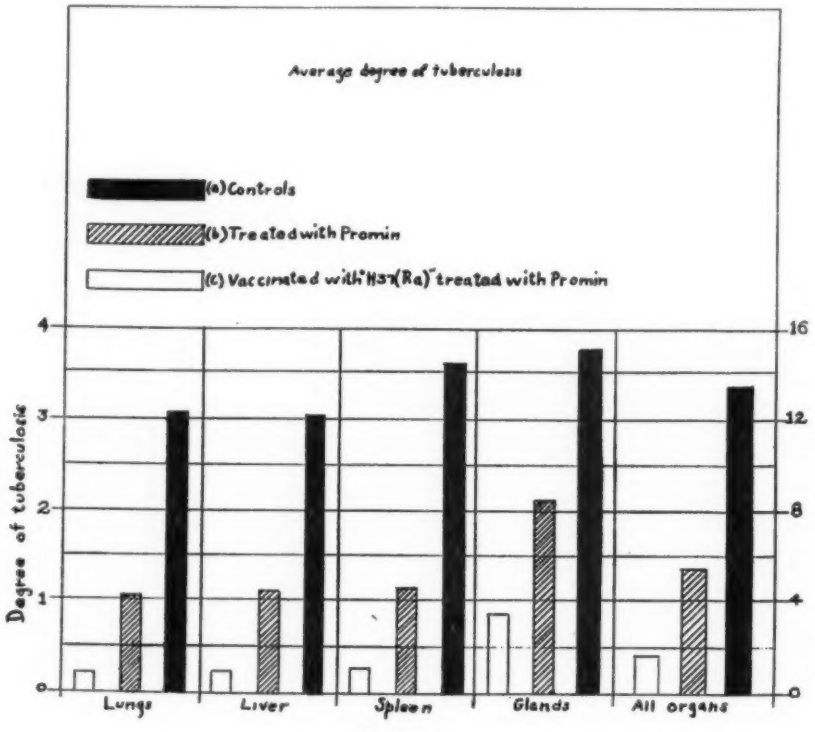

CHART 1

hundred and twenty-four days to terminate the experiment. These were found to have an average rating of *11*.

In the nonvaccinated and treated group one animal died of the infection on the one hundred and ninty-sixth day. There was generalized involvement of all organs, rating *16*. Two died, one on the thirty-eighth day and the other on the fifty-fifth day, cause of death undetermined. Ratings were *4* and *6*. One died of streptococcus pneumonia on the fifty-sixth day, rating *5*.

[3] For the purpose of record and comparison, the involvement of the spleen, liver, lungs, and lymph nodes was noted and individually assigned values were given proportionate to the extent and severity of the tuberculosis. The maximum rating of *4* in any organ was used to indicate wide-spread caseous disease diagnosed by gross inspection. The maximum value of *16* for the animal as a whole signified advanced generalized tuberculosis.

Sixteen were killed at the end of two hundred and twenty-four days. Three of the 16 had generalized involvement of the organs, rating *12* to *15*. The remaining 13 had ratings from *1* to *6*.

In the vaccinated and treated group there were no deaths from intercurrent disease or tuberculosis up to two hundred and twenty-four days, when all 20 animals were killed. Four had ratings from *3* to *9*. Nine had slight enlargement of the inguinal and iliac lymph nodes. Seven had no macroscopical disease.

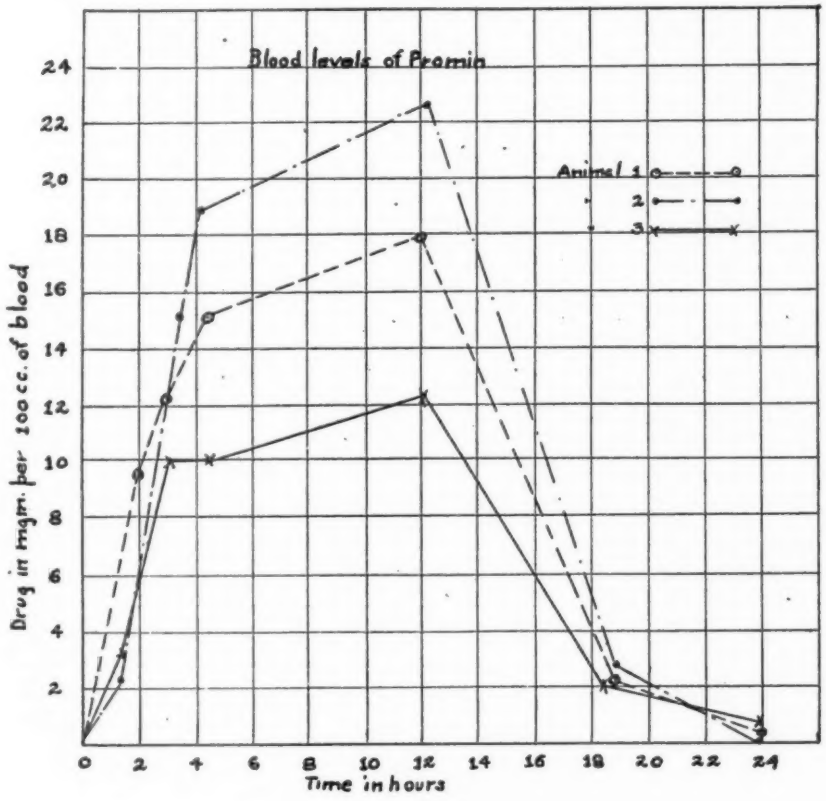
Blood levels of Promin
Animal 1
2
3
Drug in mgm. per 100 cc. of blood
Time in hours
0 2 4 6 8 10 12 14 16 18 20 22 24
2 4 6 8 10 12 14 16 18 20 22 24

CHART 2

Chart 1 is a graphic representation of the average amount of tuberculosis in the lungs, liver, spleen and nodes of the control, treated and vaccinated-treated groups of animals.

CONCENTRATION OF PROMIN IN THE BLOOD

Blood from the heart was secured from each treated animal at two weeks, two months and at the termination of the experiment. The average blood levels were found to be between 4.0 and 6.0 mg. per 100 cc. with a range of 0.3 to 13. (The oral dose was 75 mg. twice daily.)

Three normal pigs were given 200 mg. of promin orally twice daily for two

weeks. Blood from the heart was taken two and one-quarter hours after the last dose. All 3 had blood levels of 4 to 8 mg. per 100 cc. Three other normal guinea pigs were fed the same dose of promin for three weeks and blood secured *one hour* after the last dose had been given. They had blood levels of from 1.4 to 2.4 mg. per 100 cc. To determine the rate of absorption and elimination of the drug, 3 normal guinea pigs of identical weight, age and sex were fed a single dose of 300 mg. by mouth. Blood from the heart was removed at intervals up to twenty-four hours and the promin levels determined.

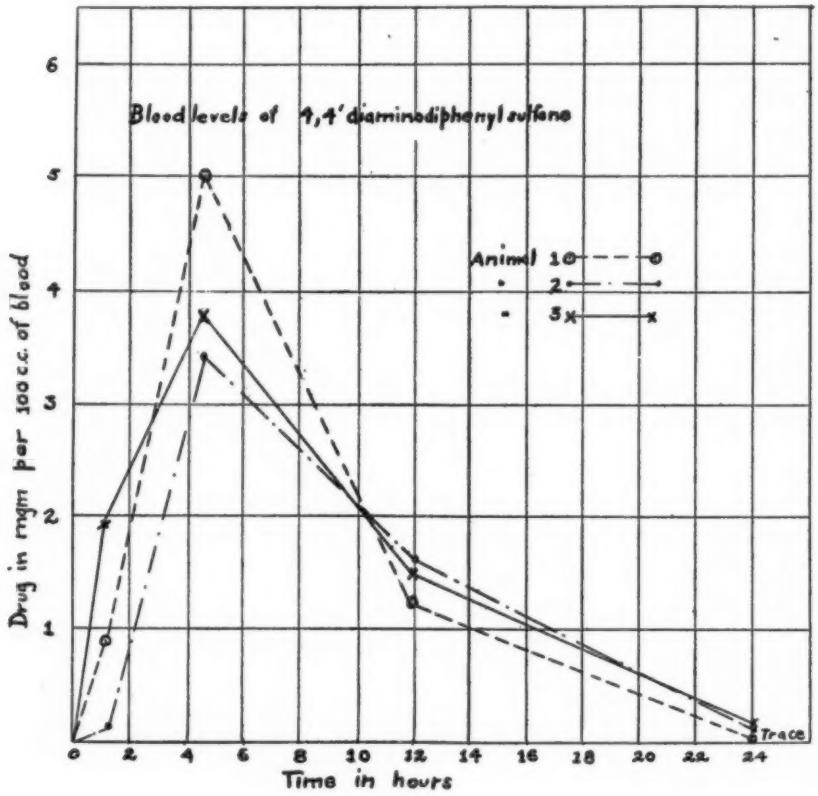

CHART 3

The results are shown graphically in chart 2. The levels of all 3 animals varied, but the curves all have the same general contour. There was a rapid rise for the first four hours, followed by a gradual rise up to twelve hours and then a tapering off until, at twenty-four hours, there remained but a trace of the drug. The maximum levels varied between 12 and 22.5 mg. per 100 cc. of blood.

The same properties of the parent drug, diaminodiphenylsulfone,[4] were studied in a similar way in 3 normal guinea pigs. After a single oral dose of 50 mg. of

[4] Diaminodiphenylsulfone was kindly supplied by the Abbott Laboratories, North Chicago, Illinois.

this drug the blood levels rose rapidly for about five hours to a peak of 3.4 to 5.0 mg. per 100 cc. and then fell to 1.2 to 1.6 at twelve hours, with only a trace remaining at twenty-four hours. The results are shown in chart 3. It will be observed from these experiments that the blood drug level obtained depends somewhat on the elapsed time between the last feeding and the withdrawal of the blood sample.

TOXIC EFFECTS OF PROMIN

No evident toxic manifestations were noted in the treated animals. In order to study more fully the toxic effects of promin, 5 normal guinea pigs were fed 150 mg. twice daily and blood counts were made at frequent intervals for thirty-five days. All animals showed a moderately severe haemolytic type of anaemia. For the first ten days there was a rapid fall in the red cells and haemoglobin with a corresponding rise in the reticulocyte count. This was followed by a secondary rise in the red cells and haemoglobin and a decline of the reticulocytes, until the sixteenth to the twentieth day. These changes were accompanied by the appearance of many nucleated red cells which, together with the increased reticulocytes, probably indicated an attempt on the part of the bone marrow to compensate for the destructive effects of the promin.

After the secondary rise there was a gradual fall in the blood count with final stabilization at the lower level. The average loss in haemoglobin at the end of thirty-five days was 4.4 g., the average drop in red cells 1.6 million. The reticulocytes rose from 1.6 per cent to 12.5 per cent. The leucocyte count varied considerably, the only significant change being a definite rise during the first few days of feeding. There were no constant changes in the differential leucocyte counts. The blood of these 5 animals was very dark and sulfhaemoglobin was demonstrated spectroscopically.

A dose of 600 mg. twice daily proved to be fatal to 3 guinea pigs within five days.

The toxicity for guinea pigs of the parent drug, diaminodiphenylsulfone, was tested in the same manner on 2 normal animals. The dose was 50 mg. per pig per day and was administered by mouth in small capsules. The capsules were inserted into the pharynx with the aid of a suction cannula; when the capsule was in place the suction was released and the capsule in turn was swallowed by the animal. (The dose was about one-third the amount of promin employed, since diaminodiphenylsulfone constitutes one-third by weight of the molecule of promin.) The same type of haemolytic anaemia, with blood count curves similar to those noted with promin, was observed. After twelve days of feeding 100 mg. per day, the average loss of haemoglobin was 4.6 g. and of red cells, 1,900,000.

CLINICAL OBSERVATIONS

In the control and treated groups of animals small caseous nodules, with an increase in the size of the inguinal nodes, were noted at the site of inoculation at the end of a month. The nodes in the control animals became progressively enlarged until they ulcerated (two months) to the surface of the skin, and these

ulcerated areas with marked enlargement of the nodes persisted until death. The nodes in the treated animals were enlarged but not to the extent observed in the control animals and in only one animal did ulceration occur. The vaccinated-treated group of animals showed only a slight enlargement of the nodes at the site of inoculation.

It was also noted in the promin treated animals that the tuberculin reactions were very pale as compared with the control animals, and that the treated and vaccinated-treated groups of animals gave positive skin tests when tested with 5 per cent OT prior to terminating the experiment.

All animals in the three groups gained in weight up to the end of four and a half months. From then until the experiment was terminated the controls gradually lost weight, whereas the treated and vaccinated-treated animals continued to gain.

SUBINOCULATION

Several guinea pigs were inoculated with tritruated lymph nodes, spleen, liver and lung tissue, removed at autopsy from the treated and vaccinated-treated animals. These subinoculations were made only from animals that had no gross evidence of tuberculosis, or from animals that had only slight enlargement of the inguinal nodes at the site of inoculation. In only one transfer animal did we fail to produce tuberculosis on subinoculation, and it was from one of the animals of the vaccinated-treated group.

DISCUSSION

The results reported in this paper confirm in part some of the observations of Feldman *et al.*, in that promin, when given orally, exerts a definite retarding effect on the progression of tuberculosis in guinea pigs. The question may then be raised as to the relative therapeutic efficacy of promin given orally as against parenterally. It would seem that the oral route is much more effective but, at the same time, more toxic.

As recently suggested by Steeken and Gardner (8), vaccinated guinea pigs were used in this experiment as well as nonvaccinated controls and non-vaccinated-treated groups. We believe, as they do, that the use of previously vaccinated animals makes for closer parallelism between the experimental conditions and those encountered in the clinical disease in humans, for the vast majority of humans who develop clinical tuberculosis have already been sensitized by previous contact with the tubercle bacillus. Previous experience with similarly vaccinated animals has shown us that this procedure alone confers a definite degree of protection against subsequent infection with virulent bacilli.

No definite toxic effects from the drug were noted in the treated animals. A more detailed study of the drug in normal guinea pigs showed that a moderate anaemia of the haemolytic type is produced by the oral administration of promin, and the blood of these animals contained sulfhaemoglobin. With the dosage employed the animals were able to compensate satisfactorily for the blood destruction. This was apparent in that many of the animals under treatment

survived two hundred and twenty-four days without any gross noticeable toxaemic effects, such as loss of weight, inability to eat, ruffled hair or marked shaking. An adequate blood level of promin can easily be maintained by feeding an aqueous solution directly into the pharynx twice daily to each animal by means of a syringe. By feeding in this manner one is certain that each animal receives the exact amount of the drug. It has been suggested that promin is hydrolyzed in the body to produce the parent substance, diaminodiphenylsulfone. This latter drug has been found to possess a greater *in vitro* (Smith, Emmart and Westfall (7), Steenken and Heise (9)) and *in vivo* (Feldman (10)) action against the tubercle bacillus than promin. We found that diaminodiphenylsulfone, when fed to guinea pigs, produced comparable blood destruction and drug levels in the blood as does promin.

It is also evident that the organisms recovered from the so-called benign nonprogressive lesions produced rapid spreading disease when subinoculated into healthy guinea pigs, except in one case and that was a specimen obtained at autopsy from a vaccinated-treated animal. It is possible that we might have obtained subinoculation tuberculosis from this animal had we triturated and concentrated all the lung, liver, spleen and lymph nodes. Observations such as these would indicate that the organisms were still present in the lesions but in a static state. Whether these lesions would have progressed and the animals died of tuberculosis had the drug been discontinued, is open to question. Again it may be that the few remaining tubercle bacilli developed a resistance to the drug and, had the drug been discontinued, there might possibly have been a marked proliferation of the already established disease. However, it is possible that the animal body may have developed sufficient resistance from the infection, so as to prevent an extension of the already established disease, or perhaps tissue alterations may have been brought about by the drug so as to exert a localizing effect upon the tubercle bacilli.

CONCLUSIONS

1. Promin, when fed by mouth, exerts a definite retarding effect on experimental tuberculosis in guinea pigs.
2. Vaccination with attenuated living tubercle bacilli combined with treatment with promin induces a greater inhibition on the progress of the disease than promin treatment in the unvaccinated animals.
3. Subinoculation tuberculosis was produced from animals treated with promin, although these animals showed only slight enlargement of the regional lymph nodes.
4. Adequate blood levels of promin and diaminodiphenylsulfone may be maintained by oral administration of these drugs.

CONCLUSIONES

1. Suministrada por vía bucal la promina ejerce un efecto retardador bien definido sobre la tuberculosis experimental del cobayo.
2. La vacunación con bacilos tuberculosos vivos atenuados, combinada con la

prominoterapia, evoca una inhibición mayor de la evolución del mal que la prominoterapia en los animales sin vacunar.

3. Se produjo tuberculosis de subinoculación partiendo de los animales tratados con promina, aunque sólo revelaban ligera hipertrofia de los ganglios linfáticos regionales.

4. Mediante la administración bucal de promina y de diaminodifenilsulfona pueden mantenerse cifras sangúíneas adecuadas de dichas drogas.

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AMERICAN TRUDEAU SOCIETY

Report of the Committee on Undergraduate Medical Education

Dr. J. L. Wilson, *Chairman*
Dr. J. B. Barnwell — Dr. Sidney J. Shipman
Dr. C. Howard Marcy — Dr. G. G. Ornstein
Dr. Frank L. Jennings

The Committee on Undergraduate Medical Education was unable to meet in 1942, or prior to the annual meeting in St. Louis in 1943, due to the pressure of work upon its members, difficulty in transportation and the upset condition of medical schools undergoing changes as a result of the war. Correspondence was maintained with the members of the Committee. Dr. Frank L. Jennings was appointed to fill the vacancy created by Doctor Casparis' untimely death. A meeting was held in St. Louis on May 5, 1943 to assemble data and to plan further work. The two members who were unable to attend had submitted studies of various medical schools on the Pacific Coast and in New York City. Doctor Barnwell reported on a visit to the University of California School of Medicine.

It was decided to take advantage of the opportunity afforded by the meeting in St. Louis to visit a local medical school. The Committee interviewed the Dean of Washington University School of Medicine and talked with several key-men in the teaching of tuberculosis during the course of the tour of that school. This method of studying the facilities and problems of a school in a group was so well received and so productive of information, that we consider it definitely superior to either questionnaires or individual visits. To avoid prohibitive expense in money and in the time of the Committee members, it is proposed that Committee members, attending medical meetings in all parts of the country during the year, visit the medical schools in or near the centre in which the meeting is held. If not more than one member of the Committee be present, he may be empowered to ask other teaching members of the American Trudeau Society to assist the Committee by accompanying him on a friendly inspection of the school or schools at hand. The data thus obtained are to be transmitted to the Chairman of the Committee for analysis at a meeting not later than April, 1944.

The response of the medical schools thus far to this study justifies its continuance. It is hoped that valuable suggestions for the improvement of teaching of tuberculosis and its better integration may be evolved to help the Deans, Curriculum Committees and others responsible for the education of medical students, during and after the war.

AMERICAN TRUDEAU SOCIETY

Report of the Illinois Trudeau Society

On May 18, 1943, the Illinois Section of the American Trudeau Society held its first meeting in conjunction with the Chicago Tuberculosis Society at the Municipal Tuberculosis Sanitarium in Chicago. About sixty persons were present.

Doctor Sweany presented fresh postmortem material from four autopsies done that morning. He pointed out several very interesting pathological conditions found in these posts.

The group then adjourned and gathered immediately after in the operating room of the Municipal Tuberculosis Sanitarium. Dr. John Barnwell of the University of Michigan presented a very interesting and instructive paper on *Tuberculosis in Student Nurses and Case Finding in a General Hospital.* The paper was presented with lantern slides.

Doctor Davison presented a paper on extrapleural pneumothorax. He presented all the patients now at the Municipal Tuberculosis Sanitarium who have had extrapleural pneumothorax. The clinical history was read and the patient's X-rays shown. This proved to be very instructive and outlined in a definite way his indications for extrapleural pneumothorax and the results obtained from the procedure. He also presented statistics on cases that have had extrapleural pneumothorax and have since been discharged from the Sanitarium.

The papers by Doctor Barnwell and Doctor Davison resulted in lively discussions.

Following adjournment, the Executive Committee of the Illinois Section of the American Trudeau Society met and made plans for a meeting next fall. Membership, Program and Nominating Committees were appointed as well as a Committee on Arrangements for the annual meeting at Decatur in the autumn.

The Committees appointed are:

Membership

Dr. W. J. Bryan, Rockford, *Chairman*

Dr. Otto Schlack, Oak Forrest — Dr. K. G. Bulley, Aurora

Nominating

Dr. H. C. Sweany, Chicago, *Chairman*

Dr. Jerome Head, Chicago — Dr. F. M. Meixner, Peoria

Program

Dr. Richard Davison, Chicago, *Chairman*

Dr. A. S. Webb, Wheaton — Dr. Charles K. Petter, Waukegan

Arrangements

(For the annual meeting)

Dr. David F. Loewen, Decatur, *Chairman*

Dr. Robert K. Campbell, Springfield — Dr. L. L. Collins, Ottawa

Following this meeting all present were guests of the Municipal Tuberculosis Sanitarium for dinner. Dr. Frederick Tice, President of the Sanitarium Board of the Chicago Tuberculosis Sanitarium, related some of the newer policies that this Board has instituted in its effort to control tuberculosis in Chicago and gave some of the excellent results which have been obtained through the introduction of these policies.

Dr. Leo M. Czaja, General Superintendent of the Municipal Tuberculosis Sanitarium, gave a short address of welcome.

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